Dissertation

The ecology and genetics of central and peripheral populations of *Carduus defloratus*

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The ecology and genetics of central and peripheral populations of *Carduus defloratus*

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CHAPTER 1

General introduction

GENERAL INTRODUCTION

This thesis tests several predictions of the abundant centre model (ACM, Brown 1984, Sagarin & Gaines 2002) by comparing central and peripheral populations of the model species *Carduus defloratus* across its Central European distribution. Analyses of continuous changes in population size and density, and in trait means from the centre towards the periphery of a species' distribution are rare, but are important in order to test the predictions of the ACM (Gaston 2009).

In contrast to earlier studies, in this thesis patterns of genetic variation in both quantitative and molecular genetic traits are investigated to analyse the importance of genetic drift and selection for population differentiation across a species range. The studies on molecular and quantitative genetic variation and structure of *C. defloratus* are combined with investigations of plant performance under standard conditions, and of the population structure and demography of the species.

The abundant centre model

Biogeographical models predict that the suitability of the environment for a species decreases with the distance to the centre of a species' geographical range (Lawton 1993, Dorken & Eckert 2001, Sagarin & Gaines 2002). Because of unfavourable conditions, peripheral populations are expected to be more isolated from each other (greater interpopulation distances), to be smaller and to be less dense than central populations. This biogeographical model of patterns in abundance is widely accepted (Hengeveld & Haeck 1982, Brown 1984, Lawton 1993, Brown et al. 1995, Hampe & Petit 2005, Samis & Eckert 2007) and has been termed the "abundant centre model" (ACM; Sagarin & Gaines 2002). Further predictions for peripheral populations derived from the model include lower reproduction of organisms, higher temporal variability of demographic transitions and of population growth rates, higher genetic differentiation among populations and lower within-population genetic diversity (Sagarin & Gaines 2002, Vucetich & Waite 2003, Eckert et al. 2008).

Geographical variation in abundance, reproduction, seed predation

Although the ACM is well founded in ecological theory and makes intuitively sense, its generality has been challenged and reviews of empirical studies on various groups of organisms concluded that there was only partial support for the predictions of the ACM with regard to patterns of abundance (Sagarin & Gaines 2002, Sexton et al. 2009). A problem in

testing the predictions of the ACM was that most studies did not adequately sample the range of species, but instead only compared a few peripheral with a few central populations. In particular, the range edges were severely underrepresented (Sagarin & Gaines 2002, Samis & Eckert 2007, Sexton et al. 2009). Very few studies on plants have tested the prediction that peripheral populations exhibit lower abundance than populations at the centre of a species' distribution range and their results have been conflicting. While some studies confirmed the predictions of the ACM (Hengeveld & Haeck 1982, Durka 1999, Jump & Woodward 2003), others did not (Carter & Prince 1988, Ribeiro & Fernandez 2000, Samis & Eckert 2007). In the annual plant species *Hornungia petraea*, population densities were actually higher at the periphery than at the centre (Kluth & Bruelheide 2005a). Moreover, Gaston (2009) has recently argued that it is necessary to differentiate between an "abundant-centre" hypothesis, which predicts that abundance declines continuously from the core to the periphery of a geographic range, and a "rare-periphery" hypothesis that predicts that abundance is low at the range limit with no wider pattern. These two hypotheses can only be tested by studying populations across the distributional range.

The factors responsible for a reduced reproduction in peripheral populations have rarely been studied. Reproduction at the margin of a species' range may be reduced because of strong resource limitation of reproduction, or due to harsh abiotic conditions that prevent seed ripening (Pigott & Huntley 1981, Reinartz 1984), or because peripheral populations are small and thus not attractive for pollinators resulting in pollen limitation of reproduction (Sih & Balthus 1987, Groom 1998). In a study on pollinator service and pollen limitation across the distribution of *Clarkia xantiana* (Moeller et al. 2012) the observed elevated pollen limitation at the geographical range limit of the plant was due to a gradual decline in the availability of generalist visitors towards the range edge of the distribution of the species. Small size of peripheral populations may also result in increased self-pollination or biparental inbreeding (Eckert et al. 2006), and in reduced seed production as an expression of inbreeding depression early in the life-cycle (Karron et al. 1995, Fischer & Matthies 1998a, Routley et al. 1999). However, several mechanisms may reduce the impact of these processes on reproduction. Small and isolated peripheral plant populations are more difficult to find for specific seed predators, and small populations of herbivores face a greater risk of extinction (Alexander et al. 2007, Holt & Barfield 2009), which may reduce seed predation in small plant populations and mask the effects of pollen limitation (Colling & Matthies 2004). Reduced opportunities for outcrossing in peripheral populations may select for alleles in outbreeding species that increase autonomous autogamy to assure reproduction (Lloyd & Schoen 1992, Herlihy &

Eckert 2005, Moeller 2006). This is supported by the fact that self-fertilising populations have been observed at the geographical periphery of outcrossing species (Solbrig & Rollins 1977, Busch 2005). Finally, small isolated populations may be less likely to exhibit strong inbreeding depression because of the purging of deleterious recessive alleles (Byers & Waller 1999), but on the other hand, inbreeding depression might be expressed more strongly in the harsher environment at range margins (Ellstrand & Elam 1993, García et al. 2000, De Vere et al. 2009). Stronger inbreeding depression and higher environmental stress might result in decreasing developmental stability. A widely used measure of decreasing developmental stability is fluctuating asymmetry (FA) which should increase when developmental stability decreases (Palmer & Strobeck 1986; Freeman et al. 1993, Markow 1995). Studies on increasing fluctuating asymmetry of central and peripheral populations are rare. Higher FA in peripheral than in central populations has been found in petals of *Lychnis viscaria* (Siikamäki & Lammi 1998) and in leaves of two *Quercus* species (Cornelissen & Stiling 2010).

Demography of central and peripheral populations

Declining environmental favourability is expected to result in a decrease of survival, growth and reproduction towards the range boundaries of species and to increase the temporal variability in demographic transitions (Sagarin & Gaines 2002, Vucetich & Waite 2003, Kluth & Bruelheide 2005b). Because the various life cycle transitions are not independent of each other and may be density-dependent, for a thorough understanding of demographic differences between central and peripheral populations, ideally the complete life cycle of a species should be understood and integrated into a population model (Angert 2006). However, this has been rarely done. Of the life cycle processes that determine the population dynamics and density of a plant species, plant reproduction has received the greatest attention. In most studies, reduced sexual reproduction in peripheral populations of plants has been observed (Pigott & Huntley 1981, Reinartz 1984, Eckert & Barret 1993, García et al. 2000, Dorken & Eckert 2001, Jump & Woodward 2003, Angert 2006, Samis & Eckert 2007), but the pattern is not universal (Angert 2006, Samis & Eckert 2007).

The ACM predicts that peripheral populations tend to exhibit higher temporal variability in population size and empirical observations partly support this (Lawton 1993, Curnutt et al. 1996, Nantel & Gagnon 1999, Vucetich & Waite 2003). However, it has also been suggested that central populations may show greater variability of demographic rates if intrinsic rates of increase are higher (Williams et al. 2003). The very few studies on plants that have compared

the demography of central and peripheral populations do not allow general conclusions, because their results are conflicting. In a study of two perennial plants, Nantel & Gagnon (1999) reported greater variability of demographic rates in peripheral populations of *Helianthus divaricatus* and *Rhus aromatica,* and Volis et al. (2002) found lower growth in peripheral populations of *Hordeum spontaneum*. In contrast, growth rates were highest in peripheral populations of two species of *Ulex* (Stokes et al. 2004), similar population growth rates in both population types were found in *Cypripedium calceolus* (García 2010) and the temporal variation of transitions was lower in peripheral populations of the annual *Hornungia petraea* (Kluth & Bruelheide 2005b). In a study of two species of *Mimulus*, survival in peripheral populations was higher in one of the species and lower in the other (Angert 2006). Recently, Jongejans et al. (2010) have criticised that hardly any studies on plant demography have investigated populations at more than one site in different regions. In their study of three species of Asteraceae they found variation in life histories over the distribution range of the species.

Molecular genetic diversity within and among central and peripheral populations

The greater isolation and smaller size of peripheral populations, as predicted by the ACM, should lead to less gene flow among populations and stronger genetic drift, resulting in higher differentiation among populations and lower within-population genetic diversity in peripheral regions (Lesica & Allendorf 1995, Leimu et al. 2006, Eckert et al. 2008). In plants, lower molecular genetic variation in peripheral populations has been found, for instance, in *Geum triflorum* (Hamilton & Eckert 2007) and *Juncus atratus* (Michalski & Durka 2007). However, while genetic differentiation in peripheral populations of *G. triflorum* was higher, it was actually lower in *Viola* ssp. (Eckstein et al. 2006). Isolated peripheral populations of *Lloydia serotina* in Wales contained similar levels of genetic variation as central populations in the Alps (Jones et al. 2001). In a recent study genetic diversity was also similar in peripheral and central populations of the steppe grass *Stipa capillata* (Wagner et al. 2011). A recent review (Eckert et al. 2008) concluded that most of the available studies detected the expected decline in neutral genetic diversity and also found increased differentiation, but that this supposition was still poorly tested. Moreover, little is known about to what extent patterns of population genetic diversity are shaped by past climate driven range dynamics rather than by recent stochastic processes (Hewitt 2004, Hampe & Petit 2005).

The variation in population genetic diversity and differentiation across the entire range of a species will be affected by both historical and contemporary effective population sizes and gene flow (Vucetich & Waite 2003, Hamilton & Eckert 2007). By definition, historical factors influenced the population genetic structure of a species in the past and do not affect it in the present, but the genetic pattern influenced by them may still be reflected in contemporary populations (Vucetich & Waite 2003, Yakimowski & Eckert 2008). Important historical factors are, for example, range fragmentations due to glaciation and subsequent founder events during postglacial range expansion (Schönswetter et al. 2003, Meeus et al. 2012), or habitat fragmentation due to human activities in past centuries (Gibbs 2001, Storfer et al. 2007, Zhang et al. 2012). In contrast, contemporary factors affect currently the distribution of genetic diversity. The current population size and features of the demography of populations that influence effective population size or obstruct current gene flow (Yakimoswski & Eckert 2008) are examples for contemporary factors. However, most of the studies (81%) that have compared the genetic structure of peripheral and central populations did not take population size into account (Eckert et al. 2008) and few have tried to distinguish between possible historical and contemporary effects on population genetic diversity and structure (Lönn & Prentice 2002, Vucetich & Waite 2003, Hamilton & Eckert 2007, Meeus et al. 2012).

Quantitative genetic diversity within and among central and peripheral populations

Peripheral populations are often the focus of conservation efforts, because they frequently are the only representatives of a species within political boundaries (Hamilton & Eckert 2007). However, the conservation value of peripheral populations has been a matter of debate (Lesica & Allendorf 1992, 1995, Van Rossum et al. 2003, Sexton et al. 2011). Because of stochastic loss of genetic variation, such populations may have low evolutionary potential (Lesica & Allendorf 1995, Vucetich & Waite 2003, Eckert et al. 2008), and because of their small size may be more strongly threatened by random environmental fluctuations (Matthies et al. 2004). It has therefore been argued that conservation efforts should be concentrated on large populations which are mostly situated in the centre of a distribution (Lesica & Allendorf 1995). On the other hand, selection regimes in peripheral populations are likely to be different from those in central populations and together with reduced gene flow this will lead to rapid genetic divergence and local adaptation in peripheral populations. It has thus been considered by some authors that peripheral rather than central populations harbour the bulk of a species'

genetic diversity (e.g. Petit et al. 2003, Hewitt 2004, Hampe & Petit 2005). Peripheral populations may harbour important adaptations (Lenormand 2002), may facilitate shifts in the geographical distribution of a species in response to climate change (Etterson & Shaw 2001, Kramer & Havens 2009), and may even be considered essential for the survival of a species (Safriel et al. 1994). In particular, disjunct populations, i.e. peripheral populations which are situated well outside the core of a species' distribution, may have a unique gene pool (Hamilton & Eckert 2007).

Previous comparisons of the genetic diversity of central and peripheral populations have almost exclusively been concerned with variation in molecular markers and there has been little effort to test whether the geographical trends in putatively neutral variation are reflected by quantitative trait variation, which is likely to influence the adaptive potential of populations (Eckert et al. 2008, but see Volis et al. 1998). Moreover, little is known about the distribution of quantitative genetic variation within and among peripheral populations. Molecular and quantitative genetic variation are often only weakly correlated, because in contrast to variation in molecular markers which is supposed to be only influenced by drift, quantitative traits are usually also under selection (Volis et al. 2005, Leinonen et al. 2008). The role of selection as compared to drift for population differentiation can be assessed by a comparison between F_{ST}-values (or Phi_{ST}-values, Peakall & Smouse 2005, Edelaar et al. 2011) and their equivalent for quantitative traits that are under selection (Q_{ST}-values) (Steinger et al. 2002, Volis et al. 2005, Leinonen et al. 2008). There are three outcomes of such a study: (1) If $Q_{ST} > F_{ST}$, this is commonly interpreted as evidence for divergent selection and adaptation to local environments; (2) if Q_{ST} and F_{ST} are similar, genetic drift alone is thought to be sufficient for explaining the observed genetic differentiation; (3) if $Q_{ST} < F_{ST}$, this is interpreted as indicating convergent selection that favours the same genotypes at different sites (Volis et al. 2005). A recent review concluded that Q_{ST}-values are on average higher than F_{ST}-values, suggesting an important role for natural selection as a cause of population differentiation in quantitative traits (Leinonen et al. 2008).

Successful species conservation depends on protecting the genetic variability of a species. Large-scale genetic comparisons of peripheral vs. central populations are needed to evaluate fundamental predictions of evolutionary theory as well as the conservation value of peripheral populations (Hamilton & Eckert 2007). These studies should be combined with investigations of population size, density and dynamics to assess the importance of recent vs. historic processes for the patterns observed, and identify potential threats to peripheral populations (Sagarin & Gaines 2002, Angert 2006, Hamilton & Eckert 2007). In Germany, many plant

species reach the limit of their distribution and plants with peripheral populations make up a considerable part of the red data lists.

Study species

Carduus defloratus L. (Asteraceae) is a common species of rocks and in meadows on calcareous substrates in the Alps, but also occurs further north in the Swiss Jura and in Germany in several low mountain ranges (Fig. 1). Because of its disjunct distribution, its cold tolerance and its virtual restriction to permanently treeless habitats *C. defloratus* is considered to be an ice-age relict in Central Europe outside of the Alps (Lange 1996). Its pattern of distribution makes *C. defloratus* particularly suitable for testing the predictions of the ACM, because it is possible to investigate populations along a central-peripheral gradient, which avoids the problems inherent in comparing just a few central and peripheral populations (Eckert et al. 2008).

C. defloratus is insect-pollinated, but at least partially self-compatible, which should promote strong differentiation among populations (Hamrick & Godt 1996). *C. defloratus* is not yet considered to be threatened, but is included in the advance warning list of the states of Hesse (Buttler et al. 1997) and Baden-Württemberg (Breunig & Demuth 1999).

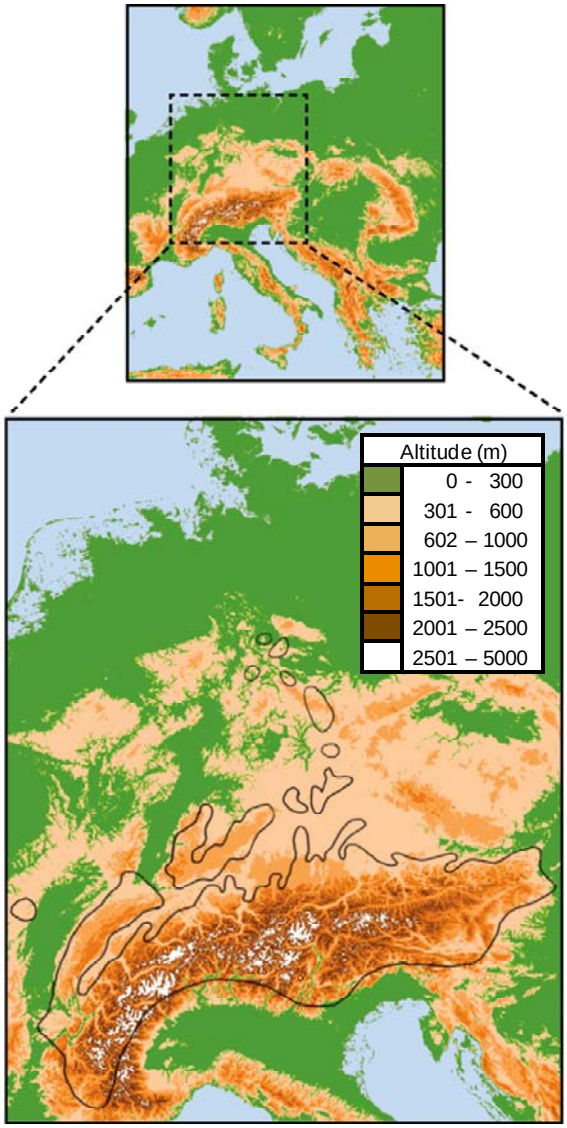

Figure 1. Map of the distribution of *Carduus defloratus* ssp. *defloratus* in Central Europe (after Meusel & Jäger 1992). The distribution area is bounded by the black line.

C. defloratus is a clonal herbaceous rosette plant. Genets spread by rhizomes which produce vegetative rosettes and flowering ramets. Ramets produce typical 1 or 2 rosettes, but sometimes more than 5 (Fig. 2). Due to its clonal growth, genets of *C. defloratus* are potentially immortal and for our study we considered ramets as individual plants. Plants flower from June to October, depending on altitude. An inflorescence (capitulum) may produce up to 200 flowers, which are insect-pollinated, partially self-compatible, but rarely autogamous. The achenes, hereafter called seeds, possess a pappus, but because of their large size (2 mg) their dispersal is very limited. In the related species *C. nutans* and *C. acanthoides* dispersal is usually less than 5 m (Skarpaas & Shea 2007).

Figure 2. *Carduus defloratus* growing in different types of habitat. (a) Plant consisting of a single rosette in a shaded habitat (b) Plant with several rosettes in an open, rocky habitat.

OUTLINE OF THE THESIS

Chapter 2 *"Abundance, reproduction and seed predation of an alpine plant decrease from the centre towards the range limit"* investigates the abundance of *C. defloratus* and several fitness related plant traits within 66 populations along an environmental gradient from the Alps in the south (central populations) towards the northern low altitudinal range limit (peripheral populations) of the species. This central-peripheral gradient was also a gradient of latitude (615 km) and altitude (342 - 2300 m). Because of the strong correlation between latitude and altitude (r = -0.80), both variables were combined into a single principle component ("centrality"). The influence of centrality, longitude and other potential variables of influence on population size, plant density, plant size, seed production, seed abortion, and seed predation, and fluctuating asymmetry of the leaves is analysed by multiple regression.

Chapter 3 *"Central and peripheral populations of* Carduus defloratus *differ in their demography"* deals with the demographic basis of the geographic variation in population size and fitness related plant traits and reproduction that were studied in Chapter 2. Moreover, it investigates the extinction risk of peripheral and central populations. Over four years, demographic data were collected in 14 populations of *C. defloratus* that are a subset of the populations studied in Chapter 2. Results based on comparative analyses of matrix models of peripheral and central populations and on multiple regression analyses are presented.

Chapter 4 *"Genetic patterns of an alpine plant across its Central European distribution are in line with the predictions of the abundant centre model"* studies the consequences of range position and population size on molecular genetic diversity within and genetic differentiation between populations across the distribution of *C. defloratus.* The 66 populations studied in Chapter 2 are complemented by a further 12 populations to investigate the influence of gene flow and random genetic drift on molecular genetic diversity of AFLP-markers within and among 78 populations from the Alps towards the northern range limit of *C. defloratus*.

Chapter 5 *"Diversity and differentiation in quantitative traits of populations of an alpine plant varies along environmental gradients"* is concerned with the quantitative genetic diversity and structure and its possible determinants in populations of *C. defloratus.* The study includes offspring from 32 populations (a subset of those studied in Chapter 2, and 4) that were grown up under standard conditions in the common garden of Marburg. To evaluate the role of selection compared to drift the quantitative genetic differentiation between populations based on 14 vegetative and reproductive plant traits are compared with the mean molecular genetic differentiation among populations. Moreover, trait means are related to environmental gradients as an alternative method to detect divergent selection.

CHAPTER 2

Abundance, reproduction, and seed predation of an alpine plant decrease from the centre towards the range limit

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ABSTRACT

Biogeographic models predict that because of increasingly unfavourable and stressful conditions populations become less frequent, smaller, less dense, and less reproductive towards the range edges. These models have greatly influenced the thinking on geographical range limits and have broad implications for ecology, evolution, and conservation. However, empirical tests of the models have rarely investigated comprehensive sets of population properties. We studied population size and density and a broad set of fitness-related traits in 66 populations of the alpine thistle *Carduus defloratus* along a latitudinal (615 km) and altitudinal (2300 - 342 m) gradient from the European Alps in the south to the northern range limit in the low mountain ranges of central Germany. Regression analysis indicated that population size and plant density declined with decreasing altitude from the centre to the range margin, but plant size increased. In spite of the larger size of plants, the number of seeds produced strongly declined towards the range margin, mainly due to an increase in seed abortion. The number of flowering plants in a population influenced all components of reproduction. Plants in large populations initiated more seeds, aborted less seeds, and produced more and larger seeds per plant. The probability that seeds were attacked by insect larvae and the proportion of seeds damaged decreased strongly from the censer to the margin of the distribution. However, in spite of the much lower level of parasitisation, plants at the range margin produced far less viable seeds. Fluctuating asymmetry of leaf width, an indicator of developmental instability, was similar across the range and not related to population size.

INTRODUCTION

The "abundant centre model" (ACM) of biogeography predicts that the favourability of habitat conditions declines from the centre to the margin of the distribution of a species. Because of less suitable conditions for growth and survival, plant populations at the periphery should be smaller, less dense, and less frequent than in the centre and produce less seeds (Brown 1984, Lawton 1993, Sagarin & Gaines 2002, Jump & Woodward 2003). The ACM has greatly influenced the discussion on geographical ranges and has broad implications for ecology, evolution, and conservation (Lawton 1993, Sagarin & Gaines 2002, Samis & Eckert 2007). However, reviews of empirical studies on various groups of organisms concluded that there was only partial support for the predictions of the ACM with regard to patterns of abundance (Sagarin & Gaines 2002, Sexton et al. 2009). However, it has been suggested that methodological problems may have contributed to this lack of support, as many studies did not sample populations from the full range of a species, but instead only compared a few peripheral with a few central populations. In particular, the range edges were severely underrepresented (Sagarin & Gaines 2002, Samis & Eckert 2007, Sexton et al. 2009). Moreover, studies on plants were underrepresented, and rarely have all components of the life cycle been investigated. Gaston (2009) has recently argued that it is necessary to differentiate between an "abundant-centre" hypothesis, which predicts that abundance declines continuously from the core to the periphery of a geographic range, and a "rare-periphery" hypothesis that predicts that abundance is low at the range limit with no wider pattern. These two hypotheses can only be tested by studying populations across the distributional range. The aim of our study was to test the predictions of the ACM along a gradient from the core to the periphery of a plant's distribution, using a comprehensive set of fitness-related traits.

The ACM predicts that reproduction increases with abundance and is highest in the centre (Jump & Woodward 2003). In peripheral plant populations resource limitation and harsh abiotic conditions may prevent seed ripening (Pigott & Huntley 1981, Reinartz 1984). Furthermore, as peripheral populations are predicted to be smaller, reproduction may be pollen limited as small, sparse and isolated populations may not be attractive to pollinators (Sih & Balthus 1987, Groom 1998). In small populations, genetic erosion, i.e. the combined effects of increased inbreeding and genetic drift may also negatively affect reproduction (Fischer & Matthies 1998a, Keller & Waller 2002, Griffin & Eckert 2003). Patterns of seed production along a gradient from the centre to the range limit may be complicated by the fact that individual plant size and reproduction is frequently density-dependent (Kluth & Bruelheide 2005a, Samis & Eckert 2007), and that plants may be adapted to conditions at the

range margin (Gaston 2003). The ACM predicts both higher density and reproduction at the distribution centre, but negative density-dependence might result in reduced individual reproduction where a plant is most abundant.

The negative influence of small population size and low plant density on the reproductive success of peripheral plant populations may be alleviated by reduced predation of seeds, as populations of specialized seed predators also face a higher risk of extinction in small populations and isolated peripheral plant populations may escape detection by specialist natural enemies (Alexander et al. 2007, Holt & Barfield 2009). Reduced seed predation has been found to mask in some plant species the negative effects of pollen limitation or genetic erosion on reproduction in small populations (Kéry et al. 2001, Colling et al. 2004). However, there are hardly any studies on the effects of range position on seed predation (but see García et al. 2000, Alexander et al. 2007).

Stressful conditions and increased inbreeding in peripheral populations may increase developmental instability (Siikamäki & Lammi 1998). An often used measurement of developmental instability is fluctuating asymmetry (FA), the nondirectional deviation from perfect symmetry (Palmer & Strobeck 1986). At the range periphery, both environmental and genetic stress factors may together lead to increased levels of FA (Siikamäki & Lammi 1998, Cornelissen & Stiling 2010).

Most studies have investigated patterns of abundance along latitudinal gradients. However, the highest abundances of a plant species have been typically found at intermediate altitudes within the elevational range and populations growing at the lower and upper elevation limit occur under ecologically marginal conditions. This view leads to the conclusion that variation in abundance along altitudinal gradients is simply a particular case of an abundant centre distribution (Herrera & Bazaga 2008). While studies of the effects of altitudinal range position may provide another test of the ACM, such studies are very rare (but see Angert & Schemske 2005, Angert 2006, Herrera & Bazaga 2008).

We studied population size, plant density and size, reproduction, pre-dispersal seed predation, seed germination and fluctuating asymmetry in 66 populations of the alpine thistle *Carduus defloratus* L. (Asteraceae) along a latitudinal and altitudinal gradient from the high mountain range of the European Alps in the south to the northern and low altitudinal range limit of the species in the central German low mountain ranges. Temperature gradients due to latitude and due to altitude are thus negatively related for this species. We asked the following questions:

(1) Do abundance, plant size, reproduction and seed predation decrease towards the range limit? (2) Does fluctuating asymmetry increase towards the range limit?

MATERIAL AND METHODS

Study species

C. defloratus is a European species with a large altitudinal range (300-2600 m; Landolt 2003) and a distribution in central Europe from the Alps to the central German low mountain ranges (Fig. 1). It also occurs south of the Alps in Italian Appenines, farther southwest in the Pyrenees, and farther east in the Tatra mountains (Meusel & Jäger 1992). In the Alps *C. defloratus* is a common species of alpine grassland and on rocks at higher altitudes, where it covers large, continuous areas. In contrast, towards the northern range limit the species becomes increasingly rare and restricted to rocks, cliffs, and open woodlands where trees are scarce, but partially shading the populations.

C. defloratus is a long-lived herbaceous rosette plant that produces inflorescences of up to 100 cm. The plants flower from June to October and produce capitula that consist of up to 200 purple, protandrous florets. Flowers are insect-pollinated, at least partially self-compatible, but rarely autogamous. The achenes, hereafter called seeds, possess a pappus and are dispersed by wind, but because of their large mass (2 mg) they are poorly dispersed. In the related species *C. nutans* and *C. acanthoides* dispersal is usually less than 5 m (Skarpaas & Shea 2007).

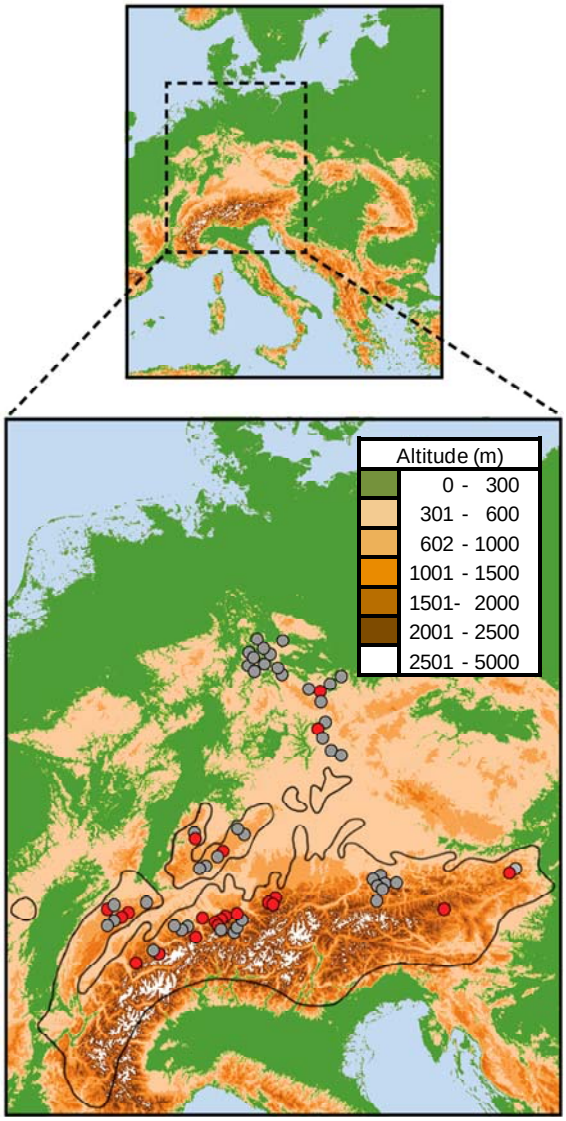

Figure 1. Map of the distribution of the alpine thistle *Carduus defloratus* ssp. *defloratus* in Central Europe (after Meusel & Jäger 1992). The distribution area is bounded by the irregular black line. The circles indicate the location of the 66 studied populations. Gray dots indicate the location of the 45 study populations from which seeds were available; red dots indicate the 21 populations from which no seeds were available.

Data collection

In summer 2006, 66 populations of *C. defloratus* were studied across the range of the species in Central Europe. The geographical distance between the southernmost and northernmost population was 615 km (see Fig. 1). However, the latitudinal gradient was also an altitudinal gradient, as in Central Europe elevation declines from the Alps towards the North German Plain. The field survey included all accessible populations at the northern low elevation range limit, a sample of those in the more southern low mountain ranges of Germany, and a sample of populations from different altitudes in the centre of the distribution of the species in the

Swiss Jura mountains and the Alps. In this system, the effects of temperature due to the gradient in latitude and those due to the gradient in altitude are negatively related. We did not study populations south of the Alps towards the southern range limit. The altitudinal range of the populations sampled was 342 - 2300 m. However, we did not sample populations at the upper altitudinal limit, because those were considered to be ecologically marginal and were difficult to locate. Also, an even sampling in the Alps was not possible due to the large number of populations.

The longitude, latitude, and altitude of each population were determined with a GPS. To study the microhabitat requirements of *C. defloratus*, we randomly selected 2 - 5 plots of 1 x 1 m in the densest part of each population. In each plot we estimated the total cover of vegetation, counted the number of flowering and non-flowering *C. defloratus* plants and measured the length of the longest rosette leaf of each plant as a measure of plant size. The height and the number of capitula were recorded for each flowering individual. Population size was determined as the number of flowering plants.

To analyze the relationship between fluctuating asymmetry (as an indicator of environmental stress) and range position, we sampled one fully developed rosette leaf from ten individuals per population, dried them between sheets of paper, and scanned them. From the scanned images the total area of the leaves and that of their left and right half were determined with the ImageJ 1.40 software (Rasband 2008). Fluctuating asymmetry (FA) was calculated as the absolute difference between the area of the left and right leaf halves, divided by the total leaf area. A relative measure of FA was used because FA increased with leaf size (Palmer & Strobeck 1986).

Ripe fruitheads were available in a subset of 45 populations, and 776 fruitheads were sampled from 10-15 plants in each population. In the laboratory, the capitula were dissected and the number of ripe seeds, aborted seeds, and of seeds destroyed by insects were counted. Three types of seeds could be clearly distinguished: healthy, viable seeds were hard and light brown, aborted seeds were much smaller and darker, and seeds attacked by insects had small holes. Aborted seeds had a much smaller mass than healthy filled seeds (0.83 mg vs. 2.06 mg). Prepupae or pupae of fruit flies (Tephritidae) were removed from dissected flowerheads, placed in Petri dishes, reared, and identified. In May 2007, the viable seeds from each plant (usually 20-40) were randomly distributed among two Petri dishes, placed on moist filter paper and stored at 20 ^{o}C. For each population, on average more than 300 seeds and overall

more than 14500 seeds were used in the experiment. Three weeks later the number of germinated seeds was counted in each Petri dish.

Statistical analysis

This study investigates population and plant properties along a cline from the centre of the distribution of *C. defloratus* in the Alps to the northern range limit. Latitude is thus a good proxy for the distance from the geographical centre of the species' distribution and has often been used for other species (Jump & Woodward 2003). However, there were also strong differences in the altitude of the studied populations, and the northern populations are at the lower limit of the altitudinal range. Because latitude and altitude of the populations were strongly negatively correlated (r = -0.80), we used principle component analysis to reduce the two geographical variables to a single principle component. This component (eigenvalue 1.73) explained 86% of the variation in latitude and altitude and increased from the north to the south and with altitude, and is thus a proxy for the centrality of a population. To investigate changes in climatic conditions along the gradient from the centre to the range margin, we extracted data (grid solution 1 km) on mean annual temperature and annual precipitation for the populations from the WorldClim database (Hijmans et al. 2005) and related them to the centrality factor. Mean annual temperature decreased (r = -0.81) and annual precipitation increased strongly (r = 0.91) with centrality.

We investigated the relationship between population characteristics, population size, plant density, centrality, and vegetation cover by multiple regressions. We calculated regressions with all possible combinations of explanatory variables and used the Bayesian information criterion (BIC) to measure the strength of evidence for each candidate model. We preferred the BIC over the Akaike information criterion, because it favours models with less parameters (Burnham & Anderson 2002). In the results we present the models with the lowest BIC. Population size, plant density and the proportion of seeds damaged by insects were log-transformed prior to analysis to achieve normally distributed residuals and homogeneity of variances. In the model for population size, plant density was not included and in the model for plant density, population size was not included, because we did not see them as explanatory variables for each other.

Relationships between variables were illustrated by simple regression plots in the case of a single significant explanatory variable, and by partial regression plots if several explanatory variables were significant. Partial regression plots show the effect of an explanatory variable

after removing the effects of the other predictors and thus adequately present the results of multiple regression analyses (Moya-Laraño & Corcobado 2008). All statistical analyses were carried out with SPSS 15.0 (SPSS Inc., Chicago, USA).

RESULTS

Multiple regression analysis indicated that of the explanatory variables only centrality influenced population size and density independently of the other variables (Table 1, Fig. 2a,b). Both population size and density increased from the periphery to the centre of the distribution.

Table 1. The effects of geographic position within the distributional range (centrality factor) and population characteristics on population size and density, and the mean size of plants in 66 populations of the alpine thistle *Carduus defloratus* in Central Europe.

Dependent variable	r^2	df	F	Explanatory variable	β	t
Log population size	0.11	64	7.49**	Centrality	0.32	2.74**
Log plant density	0.23	64	18.62***	Centrality	0.48	4.32***
Plant height	0.44	63	25.09***	Log plant density	-0.41	-3.87***
				Centrality	-0.36	-3.39**
Leaf length	0.56	63	40.31***	Centrality	-0.45	-4.74***
				Log plant density	-0.42	-4.47***

Notes: Models are presented for which the Bayesian information criterion is minimal. Potential explanatory variables for plant height and leaf length were population size and density, centrality, and vegetation cover; for population size and density, only centrality and vegetation cover. ** $p < 0.01$; *** $p < 0.001$.

Plant size measured as height and the length of the longest leaf decreased with centrality and with plant density, suggesting competition among individuals of *C. defloratus* in high density populations (Table 1, Fig. 2c-f). Fluctuating asymmetry (FA) of leaf width was not significantly influenced by centrality or any of the other variables, thus providing no evidence for different levels of stress in central and peripheral populations.

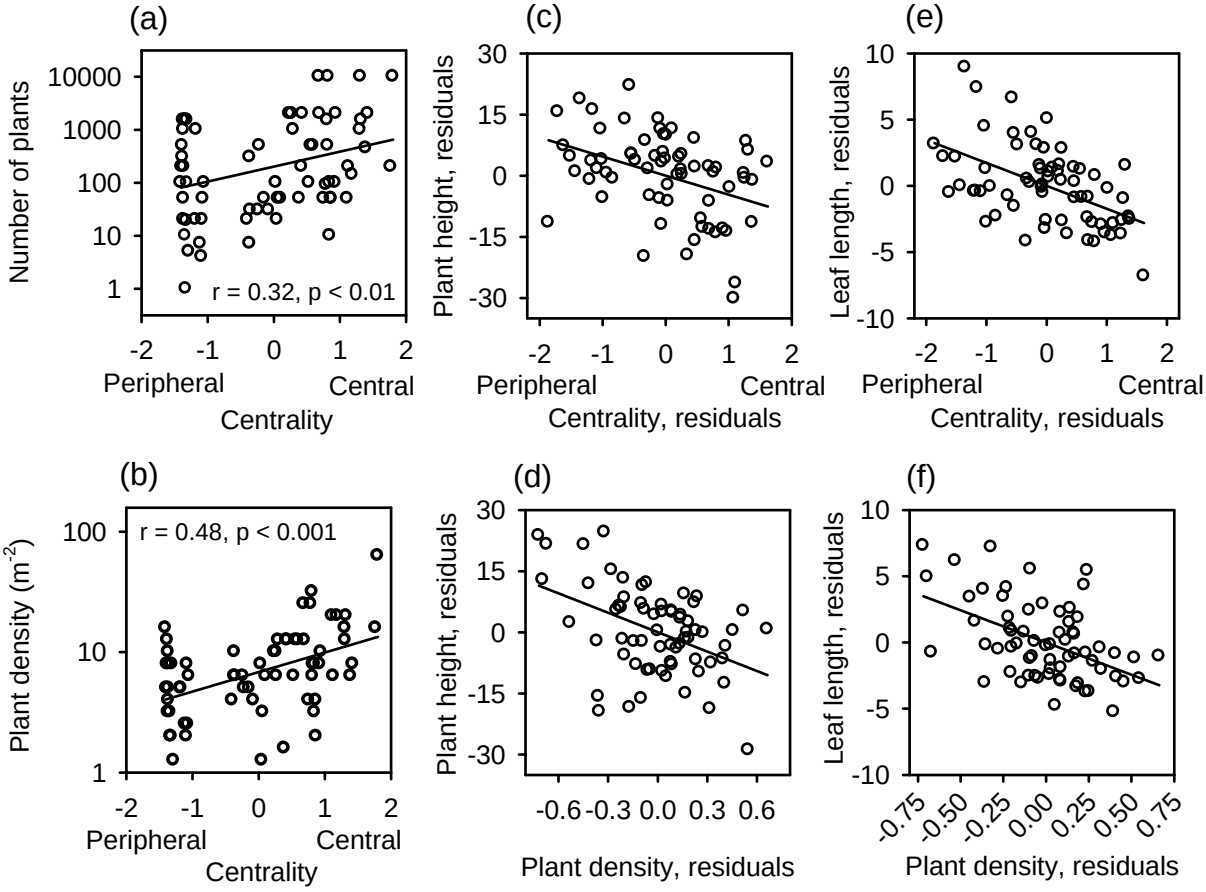

Figure 2. Relationships between (a) the size of populations, (b) population density, (c,d) plant height, and (e,f) leaf length of *Carduus defloratus* in Central Europe and significant explanatory variables. The centrality factor combines altitude and latitude and indicates the geographic position of *C. defloratus* populations within the distributional range. Shown are regression plots (a,b) in the case of single explanatory variables and (c-f) partial regression plots in the case of several significant explanatory variables. For statistical analysis see Table 1.

Range position and population size were the main independent influences on the reproduction of *C. defloratus* (Table 2). While the number of capitula produced per flowering plant was not influenced by any of the studied variables, large populations of *C. defloratus* initiated far more seeds per capitulum than small populations (Fig. 3). The number of filled, ripe seeds per capitulum increased with centrality and population size (Tab. 2). This was mainly due to a decrease in seed abortion (Fig. 4a,b), which explained 69% of the variation in the number of filled seeds.

Table 2. The effects of geographic position within the range of the species (centrality factor) and population characteristics on reproductive traits in populations of *Carduus defloratus* in Central Europe.

Dependent variable	r^2	df	F	Explanatory variable	β	t
Seeds initiated per capitulum	0.44	43	34.19***	Log population size	0.67	5.85***
Proportion of aborted seeds	0.54	42	24.75***	Centrality	-0.63	-5.92***
				Log population size	-0.27	-2.50*
Filled seeds per capitulum	0.60	42	31.46***	Centrality	0.52	5.25***
				Log population size	0.47	4.74***
Log proportion of seeds damaged	0.44	20	15.46***	Centrality	0.66	3.93***
Viable seeds per plant	0.52	41	14.62***	Log population size	0.62	5.14***
				Centrality	0.42	3.56***
				Log plant density	-0.25	-1.96
Mean mass of a seed	0.25	41	6.79**	Log population size	0.55	3.19***
				Log plant density	-0.26	-1.72

Notes: Models are presented for which the Bayesian information criterion is minimal. Potential explanatory variables were population size and density, centrality, and vegetation cover. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

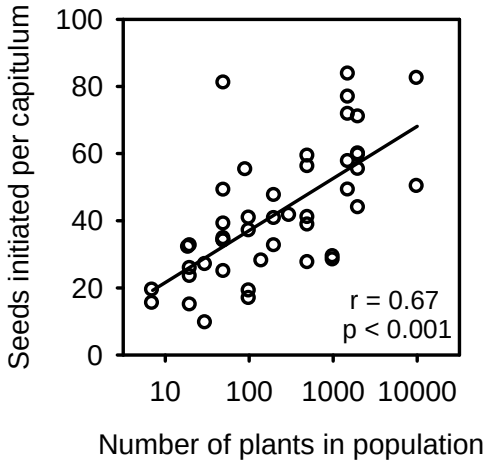

Figure 3. The relationship between the mean number of initiated seeds per captitulum in a population of *Carduus defloratus* and population size.

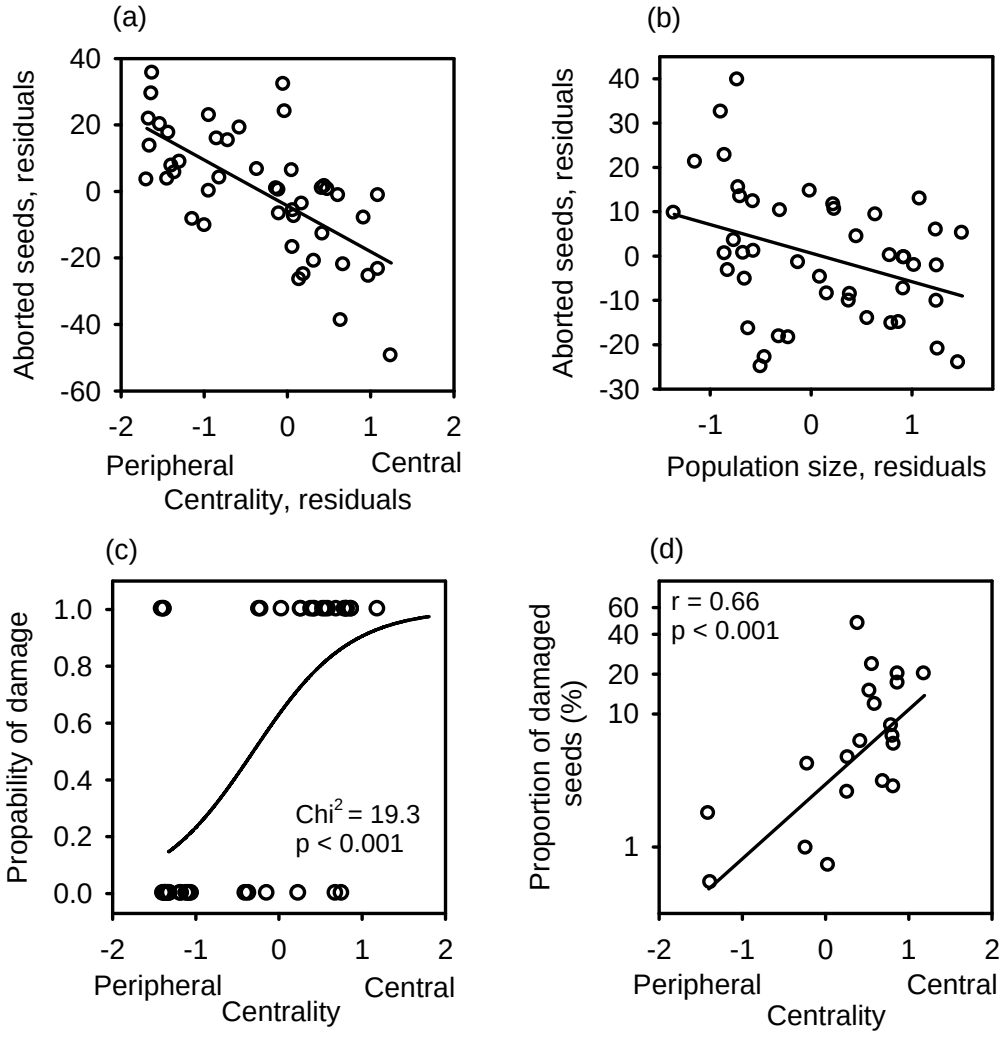

Figure 4. The relationship between (a) the proportion of aborted seeds and the geographic position of *Carduus defloratus* populations within its distributional range (centrality), (b) the proportion of aborted seeds and the size of a population, (c) the probability that at least one seed is damaged and centrality, and (d) the proportion of damaged seeds (note logarithmic scale) and centrality. The partial regression plots (a, b) show the influence of an explanatory variable after partialing out the effects of the other variable in the multiple regression model. For statistical analysis see Table 2.

In many populations of *C. defloratus* the fruitheads were attacked by a variety of insect parasites, in particular Tephritidae (*Tephritis hyoscyami*, L; *Terellia serratulae*, L.; *Urophora solstitialis*, L.*; Xyphosia miliaria,* L.) whose larvae fed on the developing seeds. Pre-dispersal seed predation varied strongly among populations from 0% - 35% of the seeds. Binary logistic regression analysis indicated that the probability that seeds were damaged at all increased significantly with centrality (Fig. 4c). The proportion of seeds damaged by insect larvae was also higher in central than in peripheral populations (Tab. 2, Fig. 4d). However, in spite of the much higher level of parasitisation, central plants produced more viable seeds.

Seed production increased also with the size of a population. In contrast to seed number, seed quality in terms of the mean mass of a seed was not significantly influenced by centrality. However, seed mass was higher in large than in small populations (Fig. 5). Seed germination was not influenced by any of the explanatory variables ($p > 0.05$).

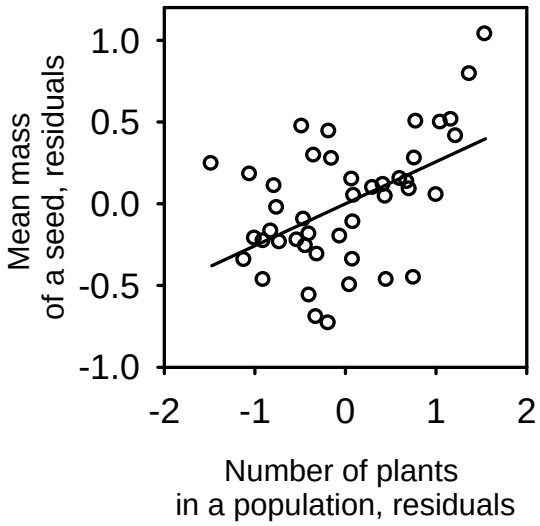

Figure 5. Partial regression plot of the relationship between mean seed mass of a seed and population size, partialing out the effect of plant density.

DISCUSSION

As in many other studies (Carey et al. 1995, Siikamäki & Lammi 1998, Durka 1999, Busch 2005, Yakimowski & Eckert 2007; but see Herlihy & Eckert 2005), peripheral populations of *C. defloratus* were smaller than central ones. In line with the predictions of the ACM, they were also less dense. This is in contrast to the results of most previous studies that found no differences in plant density between population types (Ribeiro & Fernandes 2000, Jump and Woodward 2003, Herlihy & Eckert 2005, Samis & Eckert 2007) or even higher densities at the range periphery (Kluth & Bruelheide 2005b, Yakimowski & Eckert 2007).

Contrary to the predictions of the ACM, the size of the plants of *C. defloratus* in terms of both height and leaf size was lower in central populations. This is likely a consequence of the fact that central populations of *C. defloratus* grow at higher altitudes than peripheral populations. A similar decrease of size with altitude has been found in many plant species (Woodward 1986). Altitude is a complex ecological gradient associated with a decrease in temperature and shorter growing season (Körner 2007), reduced soil nutrient concentrations (Väre et al. 1997), and increased precipitation in temperate regions (Körner 2007). Reduced partial

pressure of CO_2 may result in reduced photosynthesis and consequently in reduced plant growth and productivity at higher altitudes (Hodkinson 2005).

Reproduction of *C. defloratus* plants was much higher in central than in peripheral populations, supporting the predictions of the ACM, although plants were smaller. This was due to both a higher number of seeds initiated and reduced abortion of seeds. The lower reproduction in peripheral populations could be due to resource (De Jong & Klinkhamer 1989) or pollen limitation (Ågren 1996, Kearns et al. 1998). As plants in peripheral populations were larger, overall resource limitation is not a likely explanation for reduced reproduction. However, we cannot exclude negative effects of low water availability during seed development in the peripheral populations that grow on exposed rocks or in dry and open woodlands. Alternatively, seed production in peripheral populations may be limited by pollen quantity and quality. Populations that are small and isolated, as are many of the peripheral populations of *C. defloratus*, are often less attractive to pollinators (Byers 1995, Groom 1998) and have higher levels of inbreeding and reduced genetic variation (Ellstrand & Elam 1993, Fischer & Matthies 1998b, De Vere et al. 2009). In peripheral populations this could result in a low number of ovules fertilised (i.e. seeds initiated) and high levels of abortion due to early inbreeding depression after selfing or crossings between closely related plants (Fischer & Matthies 1998a, Colling & Matthies 2004). The importance of population size as a factor affecting reproduction in *C. defloratus* is also shown by the positive relationship between seed mass and population size.

Although reproduction in peripheral populations was much lower than in central ones, it is not known whether reduced reproduction results in lower population growth rates and contributes to the smaller size of peripheral populations. In long-lived species like *C. defloratus*, population growth rates are frequently rather insensitive to variations in seed production (Silvertown et al. 1993, Colling & Matthies 2006). However, in the medium term, lower reproduction may reduce the chance to colonize unoccupied habitat patches and thus contribute to regional rarity (Hanski & Gilpin 1991, Kéry et al. 2000).

Density-dependence of performance can result in reduced mean performance where conditions are most suitable (Samis & Eckert 2007). In *C. defloratus*, there was evidence for negative density effects on plant size and reproduction, but individual seed production was nevertheless much higher in the denser central populations, because density effects were more than compensated by the positive effects of centrality and increased population size. Higher reproduction in central plant populations is predicted by the ACM, but empirical evidence has

been conflicting. While for some species the predicted higher reproduction has been found (*Decodon verticillatus*, Dorken & Eckert 2001; *Cirsium acaule* and *C. heterophyllum*, Jump & Woodward 2003; *Juniperus communis*, García et al. 2000; *Mimulus lewisii*, Angert 2006), in others the position of populations within the range of the species did not influence seed production (*Lactuca serriola*, Carter & Prince 1985; *Cirsium arvense,* Jump & Woodward 2003; *Aquilegia canadensis*, Herlihy & Eckert 2005; *Hornungia petraea*, Kluth & Bruelheide 2005a), and even lower seed production in central populations has been observed (*Mimulus cardinalis*, Angert 2006).

The same mechanisms that may increase pollination and thus seed production in central populations at higher altitudes may also be responsible for the observed much higher levels of pre-dispersal seed predation in the centre of the distribution of *C. defloratus*. Most of the peripheral, but hardly any of the central populations escaped seed predation completely, indicating that the smaller, more isolated and less dense peripheral populations are less likely to be found by specialized insect seed predators and sustain viable populations of them. Such reductions of seed predation in small populations have been observed in several plant species (Eber & Brandl 1996, Ehlers & Olsen 2003, Colling & Matthies 2004).

Very few studies have compared seed predation in central and peripheral populations. As in *C. defloratus*, seed predation was lower in peripheral populations of *Juniperus communis* (García et al. 2000) and *Carex blanda* (Alexander et al. 2007), while there was no influence of range position on three species of *Cirsium* in Britain (Jump & Woodward 2003). However, in all those studies no information about population sizes was provided.

The higher reproduction of central, high altitude populations observed in *C. defloratus* is in contrast to the pattern observed in most other plants whose reproduction declines with altitude (Molau et al. 1989, Kelly 1998, Lord & Kelly 1999, Angert 2006). It has been suggested that this is due to pollination limitation because of reduced pollinator diversity and abundance at high altitudes (Totland 1993, Hodkinson 2005). The strong increase in seed production of *C. defloratus* with centrality indicates that these effects may be more than compensated by the higher attractiveness of the larger and denser central populations of *C. defloratus* for pollinators.

Like reproduction, pre-dispersal seed predation has been found to decrease with altitude in most plants studied (Molau et al. 1989, Spence 1990, Kelly 1998, Giménez-Benavides et al. 2008) and has been attributed to negative effects of harsher climatic conditions on insects and to smaller and less dense host plant populations (Alonso 1999, Hodkinson 2005). The

contrasting pattern found in *C. defloratus* can be explained by the increase of the density and size of its populations with altitude. Abundance and activity of specialist seed predators may depend less on changing climatic conditions along altitudinal gradients than on characteristics of the populations of their host plants (Scheidel et al. 2003).

We hypothesized that because of higher environmental stress and stronger inbreeding developmental stability would decrease and fluctuating asymmetry (FA) increase towards the range limit. However, in contrast to earlier studies on plants (Siikamäki & Lammi 1998, Cornelissen & Stiling 2010) we found no evidence for increasing FA with decreasing distance from the centre, and FA was not related to population size. There are several not mutually exclusive explanations for these findings: Plants in peripheral populations of *C. defloratus* may not be stressed more than those in central ones, or levels of FA may not be related to genetic variation or stress. However, the usefulness of FA as an indicator of genetic or environmental stress or reduced fitness is controversial (Vollestad et al. 1999, Tracy et al. 2003).

CONCLUSIONS

In conclusion, in *C. defloratus* the size and frequency of populations, plant density, reproduction and recruitment, but also seed predation, declined continuously from the core to the periphery of the species' range, thus supporting the predictions of the ACM, but not the alternative rare-periphery hypothesis of Gaston (2009). The lack of support of the ACM in other studies has been attributed to density dependence, local adaptation to conditions at the range margin, dispersal limitation, and metapopulation dynamics (Gaston 2003, Samis & Eckert 2007). In contrast, the strong support of the ACM in *C. defloratus* indicates that there is a strong decline in the suitability of the abiotic conditions and the intensity of biotic interactions for the plant from the centre to the range edge. This may be related to the fact that we studied populations along a strong gradient in climatic conditions.

In accordance with the ACM, the results suggest that reduced reproduction at the range edge plays a key role for restricting the distribution of *C. defloratus* at its northern range limit. The results indicate that the smaller size and lower density of peripheral populations may have both negative effects on reproduction because of pollen limitation and positive effects because of lower seed predation. Population size should therefore be taken into account in studies of the effect of range position on reproduction. In *C. defloratus*, strongly reduced reproduction in combination with the increasing scarcity of suitable rocky, open habitats and low dispersal

availability may restrict the species at its northern range limit. The poleward range limit of plant species' distributions has often been linked to a decrease in temperature (Pigott & Huntley 1981, Woodward 1990, Despland & Houle 1997). However, in *C. defloratus* the gradient from the centre to the northern limit of the distribution in Central Europe is a gradient of increasing temperature and decreasing precipitation. The northern peripheral populations may thus represent a receding edge in the face of climate change (Hampe & Petit 2005) and become increasingly threatened, but they might also harbour alleles important for adaptations of the species to future warmer conditions (Van Rossum et al. 2003, Petit et al. 2003, Hamilton & Eckert 2007).

CHAPTER 3

Central and peripheral populations of *Carduus defloratus* differ in their demography

in preparation
with Diethart Matthies

ABSTRACT

Biogeographic models predict that towards the range limits population growth rates decline, while the variability in vital rates and population growth rates and the risk of extinction increase. We studied the demography of the clonal plant *Carduus defloratus* over four years in 14 populations along a gradient from the centre of its distribution in the Alps towards the northern range margin in the German low mountain ranges. From the centre to the periphery altitude and precipitation decrease while latitude and temperature increase. The demographic data were analysed using matrix models. Contributions of individual transitions to spatial and temporal variation in population growth rates (λ) were studied by elasticity analyses and factorial life table response experiment analyses (LTRE). Neither λ, nor the temporal variation in λ was related to the range position of populations, but variability in both seedling survival and in the transition from vegetative to vegetative ramets increased towards the range centre. Most individual demographic transitions and their elasticities were also influenced by the range position. Progression of vegetative to flowering and stasis of flowering ramets increased, while retrogression of flowering to vegetative and stasis of vegetative ramets decreased with centrality. The importance of survival transitions increased and that of growth transitions for λ decreased towards the range limit. The LTRE analysis confirmed that similar overall growth rates of populations may mask strong differences in the contribution of individual transitions to population growth. Transitions found to be important in the LTRE were generally the same as identified in the elasticity analysis, indicating that retrospective and prospective analyses came to similar results. The strong variation in individual demographic transitions and their elasticities among populations of *C. defloratus* in different parts of its range support the view that it is not sufficient to study the demography of a species in only one region to characterise its population dynamics. However, the fact that demographic features of *C. defloratus* showed clinal variation related to gradients in centrality and thus climate, suggests that it might be possible to predict general demographic features for individual populations based on their environment.

INTRODUCTION

The abundant centre model (ACM) predicts that range limits result from increasing unfavourable ecological conditions for population growth and survival from a species' distribution centre towards its range margin (Brown 1984). Therefore, populations should become less frequent, more isolated, less dense, and should produce less seeds towards their range limits (Brown 1984, Lawton 1993, Sagarin & Gaines 2002, Jump & Woodward 2003). In contrast to the "abundant-centre" hypothesis that predicts that the abundance of a species declines continuously from its centre to the periphery following a geographic range the "rare-periphery" hypothesis predicts that the abundance is low at the range limit but does not follow a wider pattern (Gaston 2009). The underlying mechanisms for a species' range limit are still not well understood and empirical studies on various groups of organisms found only partial support for the predictions of the ACM (as reviewed in Sagarin & Gaines 2002, Sexton et al. 2009). Decreasing population densities and population size from the distribution centre of a species towards its range limit must be due to changes in demographic transitions such as survival, growth, fecundity, recruitment, or clonal growth (see Nantel & Gagnon 1999 and references therein). It has been suggested that the growth rates of populations should be lower in peripheral populations and that variation in vital rates and in growth rates should be larger at the range limit resulting in higher extinction risks (Nantel & Gagnon 1999, Willi et al. 2007). Studies analysing continuous patterns of population demographic variables along environmental gradients are needed to get a better understanding of the mechanisms shaping species' distributions (Eckert et al. 2008, Gaston 2009, Sexton et al. 2009).

However, few studies have analysed the demography of peripheral and central plant populations in detail, and in most studies less than four populations per type were compared (Nantel & Gangnon 1999, Volis et al. 2004, Stokes et al. 2004, Angert 2006, but see Kluth & Bruelheide 2005a,b). However, two recent studies (Gerst et al. 2011, Eckhart et al. 2011) used a new approach and studied continuous relationships between population growth rates and vital rates and range position. In the winter annual *Clarkia xantiana* stochastic growth rates declined among 20 populations towards its range limit, which was explained by a limited potential of the species to adapt to changing precipitation and temperature (Eckhart et al. 2011). In an experimental study of 13 winter annual plants the variance in fecundity, survival and multiplicative fitness, but not germination fraction or population density were related to geographic distance metrics (Gerst et al. 2011).

Stage-structured matrix models provide a powerful tool to reveal critical stages in the life cycle of a species and they can be used as the basis for the modelling of population dynamics (Hutchings 1991, Menges 1998, Colling & Matthies 2006). Moreover, estimation of the extinction risk for populations becomes possible if stochasticity is included in the matrix modelling (Brigham & Schwartz 2003). To investigate how very small changes in the vital rates would affect population growth (λ) elasticity analysis as a prospective approach can be used (De Kroon et al. 1986, De Kroon et al. 2000). This approach can be combined with retrospective life table response experiments (LTRE), which quantify the contribution of the actual variability in vital rates to the observed variability in lambda (Caswell 2001).

We studied the ramet demography of the clonal plant *Carduus defloratus* L. (Asteraceae) over four years in 14 populations along a gradient from the distribution centre of the species in the Alps towards the northern range limit in the German low mountain ranges. A previous study (Chapter 2) found that in line with the predictions of the ACM the size of populations, plant density, and reproduction declined from the centre to the range margin. We used stage-structured matrix models to compare the demography of central and peripheral populations of *C. defloratus* and stochastic simulations to assess their risk of extinction. Our main research questions were: (1) Are growth rates and their temporal variation in populations of *C. defloratus* related to their range position? (2) Are demographic transitions and their elasticities related to the range position of populations? (3) Which demographic transitions contribute most to differences in growth rate between central and peripheral populations? (4) Is the extinction risk higher for peripheral than central populations?

MATERIAL AND METHODS

Study species

C. defloratus is a clonal herbaceous rosette plant. Genets spread by rhizomes which produce vegetative rosettes and flowering ramets. Due to its clonal growth, genets of *C. defloratus* are potentially immortal and for our study we considered ramets as individual plants. Plants flower from June to October, depending on altitude. An inflorescence (capitulum) may produce up to 200 flowers, which are insect-pollinated, partially self-compatible, but rarely autogamous. The achenes, hereafter called seeds, possess a pappus, but because of their large size (2 mg) their dispersal is very limited. In the related species *C. nutans* and *C. acanthoides* dispersal is usually less than 5 m (Skarpaas & Shea 2007). *C. defloratus* is a common species of limestone grasslands and rocks in the centre of its distribution in the European Alps, and

populations can cover large areas. In contrast, northern peripheral populations are disjunct and restricted to isolated rocks, cliffs, and open woodland.

C. defloratus is widely distributed in the Alps, further south in the Pyrenees and the Italian Apennines, and further east in the Tatra mountains (Meusel and Jäger 1992). Within Central Europe, populations are most abundant in the Alps and Swiss Jura and become rarer further northwards in the Swabian and the Franconian Alb (Fig. 1). Very disjunct and isolated populations occur even further north in the region of the Ilm-Saale-Platte, and the most northern populations are found in the region of Werrabergland and Ohmgebirge. The altitudinal range of *C. defloratus* is from 300 m in the northern part to 2600 m in the southern part of the distribution of the species (Landolt 2003).

Data collection

In June – July of 2006, 2007 and 2008 we established multiple plots of 1 m^2 in each of eight peripheral and six central populations (Fig. 1). The number of plots varied across populations due to differences in habitat conditions and ramet density. We aimed to study at least 100 ramets per population and as many plants as possible in small populations or in very rocky habitats that are difficult to access (e.g. cliffs). We marked the four corners of each plot by metal tubes of 25 cm length. The tubes were driven deep into the soil to prevent injuries to animals, but could be relocated with a metal detector. To identify ramets in consecutive years we recorded their x and y coordinates within each plot. The plots were revisited in the summer of each year until 2011. In each year we measured the length of the longest leaf and noted whether a ramet was flowering. New ramets were recorded and also measured. Overall, we followed the fate of more than 7000 ramets. In the first two years we estimated the cover of the herb layer and used the mean value as an estimate of site productivity.

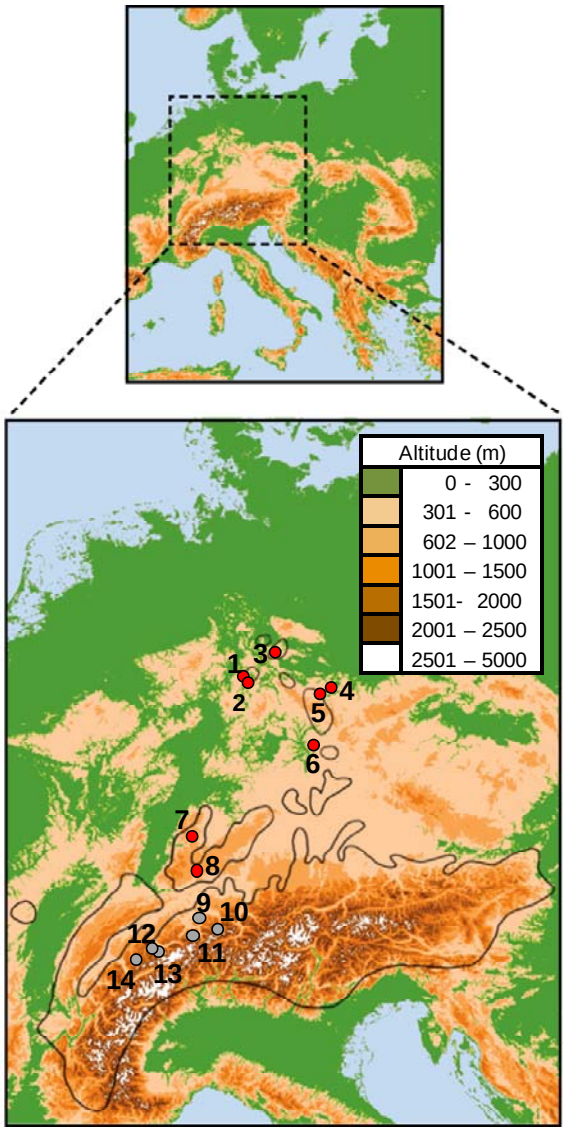

Figure 1. Map of the distribution of the alpine thistle *Carduus defloratus* ssp. *defloratus* in Central Europe (after Meusel & Jäger 1992). The distribution area is bounded by the irregular black line. The circles indicate the location of the 14 studied populations. Gray dots indicate the location of the six study central populations within the Alps; red dots indicate the eight populations within the German lower mountain ranges. The numbers indicate the studied peripheral populations within the two population types. Werrabergland and Ohmgebirge (1: HOH; 2: SAL; 3 OHM); Ilm-Saale-Platte (4: JEN; 5: REI); Franconian Alb (6: KLE); Swabian Alb (7: WEN; 8: TAL); Alps (9: GOL; 10: BRA; 11:GEN; 12: KAN; 13: OES; 14: SAN).

Statistical analysis

We investigated the demography of *C. defloratus* along a cline from the centre of the distribution of the species in the Alps towards the range margin in the northern German low mountain ranges. Towards the northern range margin latitude increases, but altitude decreases and the northern populations are at the lower limit of their altitudinal range. Because latitude

and altitude are strongly negatively correlated (r = -0.80), we used in a previous large study on plant performance in 66 populations a principal component combining the two variables as a proxy for the centrality of a population (see Chapter 2). This component explained 86% of the variation in latitude and altitude and was strongly correlated with both mean annual temperature (r = -0.81) and annual precipitation (r = 0.91; Chapter 2). As the populations in the current study are a subset of those populations, we used the same measure of centrality. To analyse demographic patterns of *C. defloratus* we used two approaches. For certain analyses we compared the demography of central and peripheral populations. In these analyses populations in the Alps were considered as central and the disjunct populations outside the Alps as peripheral. We also studied continuous changes in demographic features along the gradient from the centre to the range margin.

We distinguished three life stages: seedlings with leaves of up to 3 cm length, vegetative plants, and flowering plants. The proportion of plants in the different life stages was calculated for each population. To compare the overall population structure of peripheral and central populations we calculated the proportions of plants in different stages within each population and year (2008-2011) and averaged them. Differences in the population structure between central and peripheral populations were analysed by multivariate analysis of variance.

We calculated the mean ramet density for each population and year by dividing the number of recorded plants in a given year by the total area of the permanent plots. We assumed that there was no net dispersal of seeds into the plots. To compare the demography and dynamic of populations or population types (peripheral vs. central) of *C. defloratus*, we constructed stage-based transition matrices (Fig. 2, Caswell et al. 2001), based on pooled data from all permanent plots within a population. Ramets that were produced within 2 cm of the location where in the previous year a ramet had been present, were considered to be the same ramet, whereas other adult ramets were considered to be new ramets produced by clonal growth. The mother ramets of these new clonal ramets could not be determined and we therefore partitioned the new ramets among the vegetative and flowering ramets in proportion to their number in the previous year.

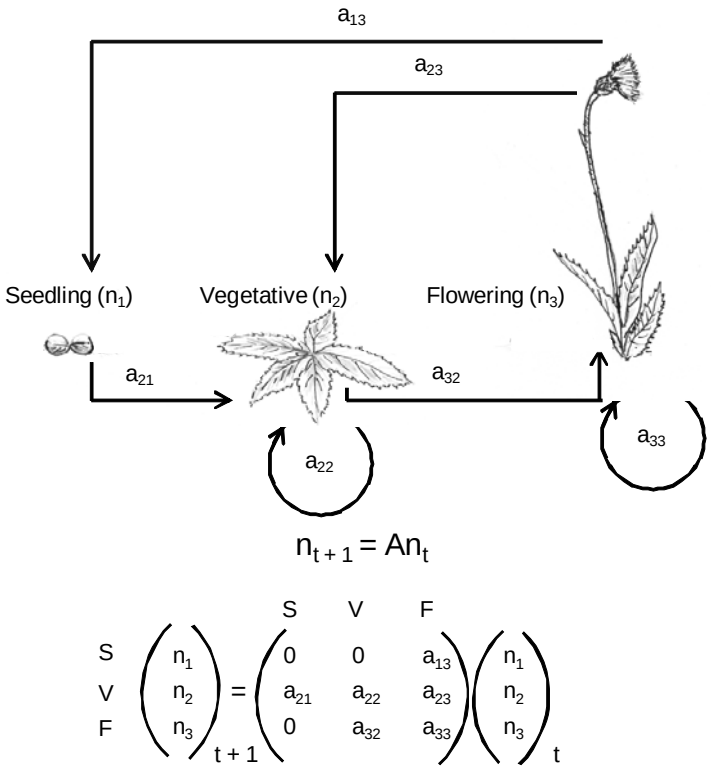

Figure 2**.** Ramet life cycle of *Carduus defloratus* with the three stages seedlings (S), vegetative (V), and flowering plants (F), and the corresponding matrix population model (s. Caswell et al. 2001).

Deterministic projection of population growth

To analyse the temporal (among transition periods) and spatial (among populations) variation in the fate of ramets we used log-linear models (Caswell 2001). To calculate finite rates of increase (λ) and its variation for each of the 14 studied populations, we constructed separate transition matrices for each combination of population and transition interval. To compare demographic characteristics between the two population types, central and peripheral, we calculated mean matrices for each type pooling all plants from the populations of each type (2008-2011). Differences between central and peripheral populations in λ were evaluated with a two-tailed permutation test. We randomly permuted individuals between the two types to obtain two pooled populations of the original size. We then constructed for each population type a matrix, calculated λ, and a test statistic $\theta^{(i)} = |\lambda_{peripheral} - \lambda_{central}|$. This was repeated 10 000 times. The probability of obtaining a θ as large as or larger than the θ observed was calculated as (Caswell 2001):

$$P\left[\theta \geq \theta_{obs} \middle| H_0\right] = \frac{\#\left\{\theta^{(i)} \geq \theta_{obs}\right\} + 1}{10000 + 1}$$

Analogous permutation tests were carried out to study differences in individual demographic transitions, temporal variability (CV) of λ, and time for seedlings to reach the flowering stage, and mean life expectancy of stages. We calculated the mean time for seedlings to reach the flowering stage and the mean life expectancy of the stages using eq. 9 and eq. 3 in Cochran & Ellner (1992), respectively. Following Ehrlén & Lehtila (2002) we subtracted one year from the estimated life spans.

Comparison of current and stable stage distribution

We compared the stable stage structure of both population types calculated from their 2008-09 mean matrices to the stage structure observed in the first year for which data were available for all populations, 2008. The difference was characterised by Keyfitz Δ, which varies from 0 (both structures identical) to 1 (maximum difference; Caswell 2001). The stable stage structure was also calculated for the mean matrices (2008-2011) for each population type. To determine the rate of convergence to the stable stage structure we calculated the damping ratios. Large values of the damping ratio (> 3, Sandercock et al. 2005) indicate that a population would rapidly approach its stable stage distribution while values close to one indicate slow convergence on the stable stage structure (Rooney & Gross 2003). The difference between population types in damping ratio was evaluated with a permutation test. In addition, we calculated the damping ratio separately for each population for the transition interval 2008 – 2009.

Analysis of elasticities and regional elasticities

To analyse the relative sensitivity λ to changes in each matrix element we calculated for each population elasticity matrices based on pooled data from 2008-2011. Elasticities of matrix elements that consisted of a clonal and a non-clonal component were calculated separately for each component (Weppler et al. 2006). First, elasticities were calculated for the sum of the components and then separately for the non-clonal component of a matrix element, varying only one element of the matrix at a time. The elasticity of the clonal components was then calculated as the difference between the total elasticity and that of the non-clonal component. The elasticity matrices were divided into regions that describe different parts of the plants life cycle and grouped elasticities for growth, survival, and fecundity calculated (Silvertown et al. 1993). We also calculated grouped elasticities for the peripheral and central types of

populations, based on the pooled data from 2008 to 2011. Differences between central and peripheral populations in elasticities were evaluated by permutation tests.

Analysis of clines in demographic characteristics

We studied the relationships between demographic characteristics of populations and the possible explanatory variables vegetation cover, centrality (i.e. position within the central - peripheral gradient), population size and ramet density by multiple regressions. To measure the strength of evidence for each possible model we used the Bayesian information criterion (BIC). The models with the lowest BIC are presented in the results. Population size and ramet density were log-transformed prior to analysis. If the most supported model included a single explanatory variable we illustrate results by a simple regression plot, whereas if several variables were included, we present partial regression plots (see Moya-Laraño & Corcobado 2008).

Life table response experiment (LTRE)

We modelled the finite growth rate (λ) of *C. defloratus* with a factorial life table response experiment (LTRE, Caswell 2001) as a linear function of the fixed factors population type and yearly transition interval, and the interaction between population type and transition interval. Transition matrices were calculated based on data pooled across all populations within each population type for each of the three transition intervals from 2008 to 2011. We used reference matrices calculated from pooled raw data and the transition rates were thus weighted by their frequency in the total data set (Horvitz & Schemske 1995, Angert 2006). This approach leads to a better approximation of the observed finite growth rates than a mean reference matrix of averaged transition frequencies (Angert 2006). To analyse which transitions contributed most to differences in growth rate we partitioned the main effects and the interaction effects into the effects of the particular transitions (Caswell 2001, Angert 2006). We obtained 95% confidence intervals by bootstrapping the data 10000 times, recalculating the statistics and determining the range of values bounded by the lower and upper 2.5% of their distribution.

Simulation of stochastic growth rates and extinction risk of populations

In contrast to deterministic analyses that assume a stable stage structure and project population growth based on a specific transition matrix, stochastic analyses take environmental variability into account. For calculations of stochastic growth rates (λ_s), we used the method of matrix sampling (Caswell 2001, Morris & Doak 2002). Population growth for each population type (peripheral and central) was simulated over 10 000 time intervals. At each time step we randomly selected one of the observed matrices with equal probability. We calculated the stochastic growth rate by back-transforming the arithmetic mean of all pairs of log $[(N_{t+1} / N_t)]$. λ_s was calculated for each population and for each population type. To compare the extinction risk of central and peripheral populations, we used stochastic simulations (1000 runs) of the population dynamics based on pooled matrices for each transition period and population type. The stage structure included in the model was the one recorded in 2008. We sampled the three matrices of the period 2008-2011 with equal probability and calculated the proportion of runs in which less than 5 plants survived.

All statistical analyses were carried out with IBM SPSS 20, while permutation tests and bootstrap calculations were carried out using dedicated scripts in R 2.12.1 (R Development Core Team 2011).

RESULTS

Population structure and dynamics

In both central and peripheral populations vegetative ramets were more frequent than flowering ramets, and seedlings constituted only a small part of all plants (<10%, Fig. 3). However, central and peripheral populations differed in their stage structure. The mean proportion of vegetative plants was higher and that of flowering plants and seedlings lower in peripheral populations, indicating less favourable conditions for both flowering and recruitment (Fig. 3). A comparison of the observed and the projected stable frequency distribution of stages based on data from the first study interval (2008-2009) indicated that the observed frequencies of the stages differed from the stable stage structure, both in peripheral (Keyfitz $\Delta = 0.235$) and in central populations ($\Delta = 0.085$, Fig. 4). While the structure of the peripheral populations appeared to be further from the stable structure, this difference was not significant ($p = 0.22$, permutation test). However, damping ratios (ρ) calculated from the mean matrices for the first study interval indicated that, if conditions stayed the same,

peripheral populations (ρ = 12.0) would converge much faster to the stable stage structure than central populations (ρ = 5.8; $p < 0.001$).

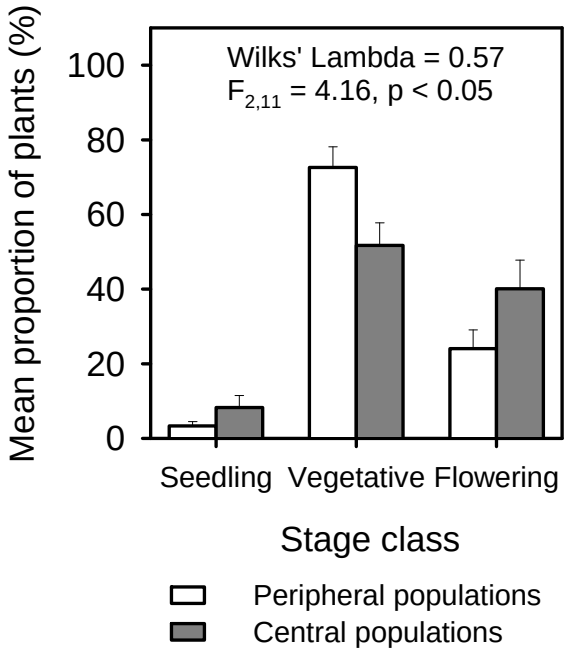

FIGURE 3. Stage structure of central and peripheral populations of *Carduus defloratus.* Means over four years (2008-2011) + 1 SE.

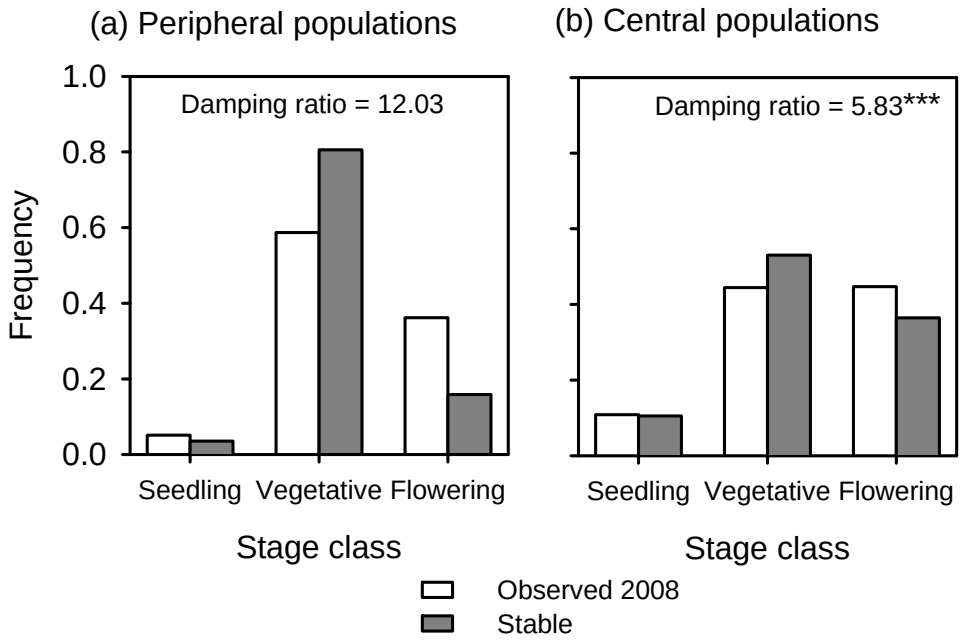

Figure 4. Comparison of the frequency distribution of stages of *Carduus defloratus* in 2008 in (a) peripheral and (b) central populations with the stable stage structure. $p < 0.001$.

Variation in the density of populations of *C. defloratus* over the study period (2008-2011) was considerable and based on mean matrices per transition interval was similar for peripheral and central populations (CV = 27% vs. 29%, $F_{1,12} = 0.29$, $p = 0.22$). There was no overall clear pattern in the dynamics of the individual populations. Over the study period, the density of some populations, both peripheral and central, increased, while that of others decreased (Fig. 5a-c). The dynamics of some peripheral populations that were geographically close (JEN, REI, TAL) were very similar and densities developed in parallel, suggesting that the same factor (e.g. regional weather conditions) may have been responsible. The dynamics of the three populations from the northern range margin (HOH, SAL, OHM) were also roughly similar. However, the two populations from the Swabian Alb (WEN and TAL) differed in their dynamics. Of the central populations, KAN and GEN developed in parallel over four years, but others did not (Fig. 5b).

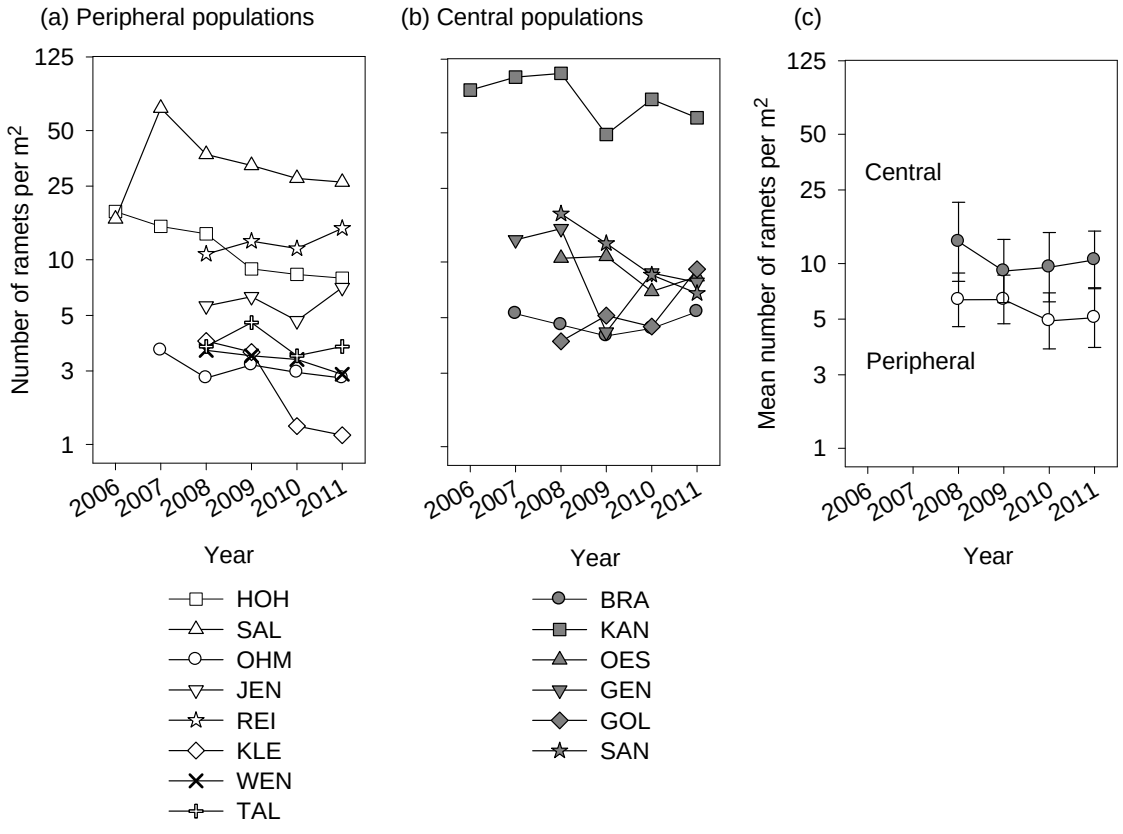

Figure 5. Dynamics of ramet density in (a) eight peripheral, (b) six central populations of *Carduus defloratus*, and (c) means for both population types. For abbreviations of population names see Table 1. Note log-scale for plant density; differences in slopes therefore correspond to differences in observed growth rates.

While in some populations the finite rate of increase varied little, in others there was considerable variation (Table 1). The strongest variation was shown by the central population GEN, where a very low growth rate in 2008-2009 was followed by strong growth in the next year. This was due to a lack of habitat management in 2008 (no mowing), followed by management in the next year. Spatial variation, i.e. among populations, was also considerable. In log-linear analyses, the fate of plants varied both over time (2008-2011; $G^2 = 39.0$, $p < 0.01$) and among populations ($G^2 = 580.7$, $p < 0.001$), and was also influenced by the combinations of population and time ($G^2 = 339.3$, $p < 0.001$).

The overall growth rate 2008-2011 for peripheral and central populations was similar (0.960 [CI 0.920 - 1.013] vs. 0.971 [CI 0.928 - 1.014]; $p = 0.72$), and not different from 1, but growth rates differed in some of the years. In 2008-09 the mean growth rate of peripheral was higher than that of central populations ($\lambda = 0.986$ vs. 0.810; $p < 0.001$, permutation test), while in 2009-10 growth of peripheral populations was lower ($\lambda = 0.861$ vs. 1.041; $p < 0.001$) and in 2010-11 it was similar to central populations ($\lambda = 1.057$ vs. 1.085; $p = 0.64$). Variation in λ among years was similar in peripheral and central populations (CV = 9.5% vs. 15.2%; $p = 0.15$). Including environmental stochasticity hardly changed growth rates of peripheral or central populations. Stochastic (λ_S) and deterministic growth rates (mean matrices 2008-2011) for the individual populations were strongly correlated ($r^2 = 0.91$, $p < 0.001$, $n = 14$) and λ_S was similar in peripheral ($\lambda_S = 0.9572$, CI 0.9206 - 0.9979) and central populations ($\lambda_S = 0.9761$, CI 0.9338 - 1.0196). Multiple regression analyses did not reveal significant relationships between λ_S, λ or the CV of λ over time of the 14 study populations and centrality, vegetation cover, population size or density.

Table 1. Finite growth rate (λ) of *Carduus defloratus* in peripheral and central populations in different years, and coefficient of variation (CV) of λ from 2008-2011. CVs in parenthesis are calculated for longer time periods, if available.

Population		Finite rate of growth (λ)					CV of λ (%)
		2006 / 2007	2007 / 2008	2008 / 2009	2009 / 2010	2010 / 2011	
Peripheral							
Hohestein	HOH	0.785	0.901	0.559	0.815	1.036	29.7 (21.4)
Salzfrau	SAL	1.785	0.892	0.846	0.929	0.977	7.2 (36.3)
Ohmberg	OHM		0.683	1.161	0.895	0.941	14.2 (21.3)
Jena	JEN			1.045	0.676	1.443	36.4
Reinstädt	REI			1.196	0.931	1.247	15.1
Kleinziegenfeld	KLE			1.112	0.500	0.970	37.2
Wenthof	WEN			0.953	0.972	0.796	10.7
Talmühle	TAL			1.023	0.820	1.092	14.5
Central							
Braunwald	BRA		1.004	0.818	1.106	1.051	15.4 (12.6)
Kandersteg	KAN	1.325	0.596	0.807	1.597	0.826	41.9 (40.3)
Oeschinen See	OES			1.040	0.648	1.177	28.7
Gental	GEN		1.133	0.284	2.125	0.930	83.9 (68.2)
Goldau	GOL			1.059	1.130	1.551	21.3
Sanetsch See	SAN			0.887	0.702	0.855	12.1
CV of λ (%)		38.6	23.0	27.1	42.6	21.0	

Analysis of transition matrices

While there were no overall differences in mean population growth rates, all matrix elements (demographic transitions) differed significantly between peripheral and central populations (Table 2a; $p < 0.01$; permutation tests), except for the probability that vegetative or flowering adults clonally produced vegetative ramets ($p = 0.23$). In both types of populations few seedlings were produced per flowering plant and most of those did not develop into a vegetative adult in the next year (Table 2a). However, recruitment of new plants through sexual reproduction was much lower in peripheral than in central populations, as less seedlings were produced and their survival was lower. In fact, in six of the eight peripheral populations, but only in one of the central ones, either no seedlings were observed over four years of study or none of them survived to become an adult. Little more than half of the vegetative ramets in both types of populations (54% vs. 53%) reappeared in the next year. In peripheral populations it was less likely that vegetative progressed into flowering ramets, and flowering ramets were also less likely to flower again. Clonal growth contributed

considerably to both vegetative and flowering ramets in both population types. Ontogenetic development of *C. defloratus* was on average slower in peripheral than in central populations. Seedlings needed 5.2 years to develop into a flowering plant in peripheral, but only 3.8 years in central populations ($p < 0.001$; permutation test) and their life expectancy was higher in the centre (1.62 vs. 1.30 years, $p < 0.05$). However, mean life expectancy of flowering ramets was higher in peripheral than in central populations (2.43 vs. 2.19 years; $p < 0.01$).

Table 2. Matrix of (a) the mean transitions and (b) their elasticities in peripheral and central populations of *Carduus defloratus.* Demographic data were pooled over eight peripheral and six central populations, respectively, and three transition intervals (2008-2009, 2009-2010, 2010-2011). Contribution of clonal growth in italics.

(a)

	Transitions		
	Seedling	Vegetative	Flowering
Peripheral	($\lambda = 0.9603$)		
Seedling	0	0	0.1586
Vegetative	0.1346	0.4629 + *0.3013*	0.3843 + 0.3013
Flowering	0	0.0778 + *0.0956*	0.2388 + 0.0956
Central	($\lambda = 0.9709$)		
Seedling	0	0	0.2791
Vegetative	0.2887	0.3682 + *0.2763*	0.2215 + 0.2763
Flowering	0	0.1642 + *0.1267*	0.3265 + 0.1267

(b)

	Elasticities		
	Seedling	Vegetative	Flowering
Peripheral			
Seedling	0	0	0.0049
Vegetative	0.0049	0.3578 + *0.2452*	0.1085 + *0.0413*
Flowering	0	0.0996 + *0.0551*	0.0506 + *0.0320*
Central			
Seedling	0	0	0.0286
Vegetative	0.0286	0.1917 + *0.2038*	0.1117 + *0.0599*
Flowering	0	0.1657 + *0.0346*	0.1093 + *0.0660*

In multiple regression analyses, most demographic transitions in the 14 study populations were related to the centrality of a site, and some to its vegetation cover and to density (Table 3, Fig. 6), while population size had no influence. The probability that seedlings developed into vegetative plants increased, while that of vegetative ramets to remain in their stage (stasis) decreased with centrality (Fig. 6a,b). Seedling survival also increased with plant density (Table 3), indicating that not intraspecific competition, but habitat quality influenced seedling survival. The probability of vegetative progressing into flowering ramets increased with centrality (Fig. 6c). This transition and the probability of a flowering ramet to flower in the next year increased with vegetation cover in a population (Fig. 6d,e), indicating a positive influence of site productivity on flowering probability. Stasis in the flowering stage tended to increase with centrality ($r = 0.48$, $p = 0.08$), but this effect was not part of the most supported model. Retrogression of flowering to vegetative ramets decreased with centrality (Fig. 6f), whereas the fecundity transition was not related to any of the potential explanatory variables (all $r < 0.08$, all $p > 0.77$).

In contrast to the temporal variability of overall population growth rate, that of some demographic transitions was related to range position. Variability in both seedling survival ($r = 0.62$, $p < 0.05$) and in the transition from vegetative to vegetative ramets ($r = 0.46$, $p = 0.10$) increased towards the range centre. All correlations between the variability of the other transitions and centrality had also a positive sign, but these relationships were far from significant ($p > 0.18$).

Table 3. The effects of geographic position within the range of the species (centrality factor) and population characteristics on demographic transitions in populations of *Carduus defloratus*. For calculations of the transitions we pooled fates within each population between 2008 and 2011. Models are presented for which the Bayesian information criterion is minimal. Potential explanatory variables were population size, ramet density, centrality and vegetation cover.

Transition	df	r^2	F	Explanatory variable	β	t
Seedling => Vegetative	11	0.46	4.61	Centrality	0.51	2.27*
				Log density	0.35	1.56
Vegetative => Flowering	11	0.67	10.94**	Vegetation cover	0.54	2.90*
				Centrality	0.46	2.45*
Vegetative => Vegetative	12	0.32	5.50*	Centrality	-0.56	-2.35*
Flowering => Flowering	12	0.49	11.66**	Vegetation cover	0.70	3.41**
Flowering => Vegetative	12	0.42	8.80*	Centrality	-0.65	-2.97*

Notes: df, degrees of freedom; *, $p < 0.05$; **, $p < 0.01$.

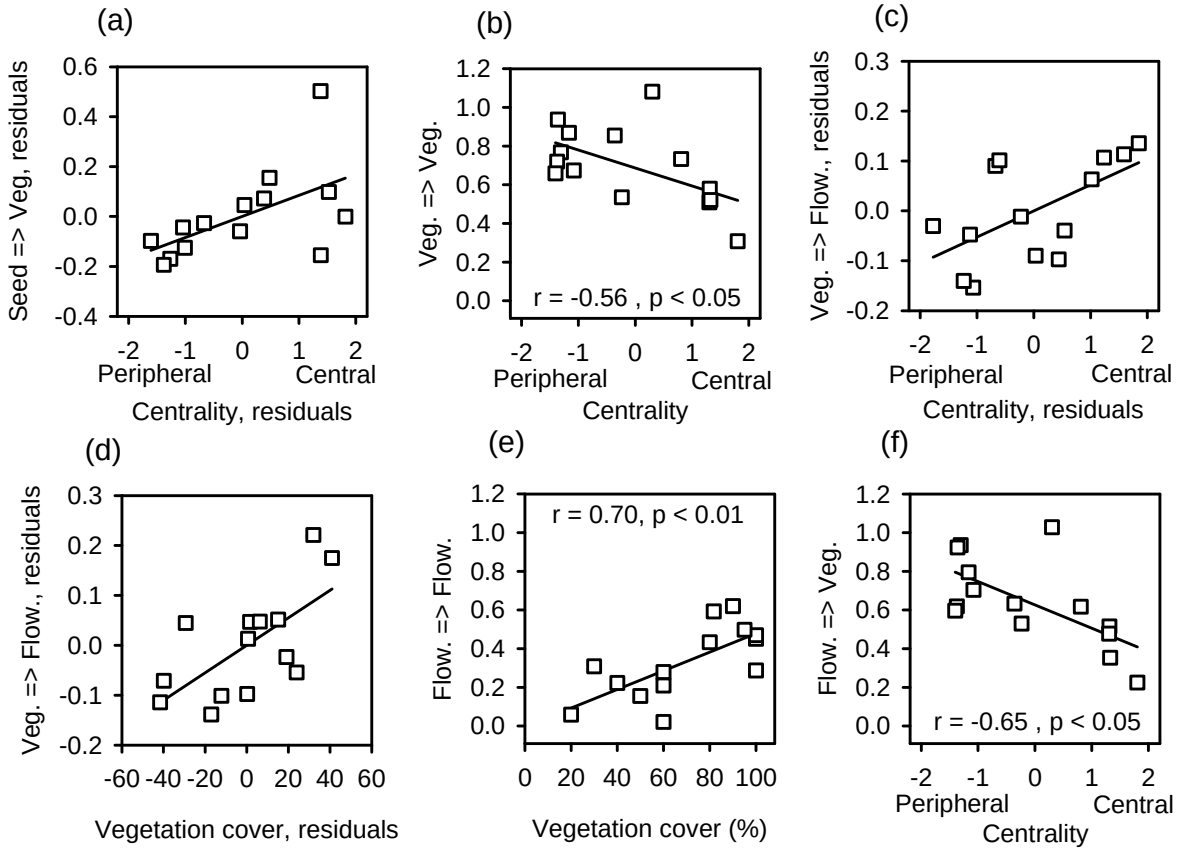

Figure 6. Relationships between the demographic transitions from (a) seedling to vegetative ramet, (b) vegetative to vegetative ramet, (c, d) vegetative to flowering ramet, (e), flowering to flowering ramet, and (f) flowering to vegetative ramet of *Carduus defloratus* and significant explanatory variables. Shown are partial regression plots (a, c, d) in case of several explanatory variables included in the most supported model, and regression plots (b, e, f) in the case of single explanatory variables. For statistical analysis see Table 3.

Elasticities

The elasticities of all transitions in mean matrices differed significantly between peripheral and central populations ($p < 0.01$, permutation test). In both types of populations changes in the proportion of vegetative adults remaining vegetative had the strongest effect on the population growth rate (λ), but the influence was much stronger in peripheral populations (Table 2b). In contrast, the elasticity of the transition of vegetative to flowering ramets was lower in peripheral populations. In both population types the effect of changes in clonal growth (sum of all clonal elasticities) was much lower than that of changes in growth, stasis, and retrogression of ramets (peripheral: 0.62 vs. 0.37; central: 0.58 vs. 0.36). The elasticity of transitions involving seedlings was low in both population types, but in particular in peripheral populations. Regression analyses indicated a significant decrease in the elasticity of

the stasis of vegetative ramets ($r = -0.66$, $p < 0.01$) and an increase in the elasticity of the stasis of flowering ramets ($r = 0.63$, $p < 0.05$) with the centrality of populations.

A triangular ordination of populations according to their grouped elasticities indicated that peripheral and central populations were all characterised by low contributions of fecundity (eF) (Fig. 7). Greater variation was found in the contributions of growth (eG), and particularly survival (eL). The contributions of both growth and survival to the growth rate of a population were related to its position within the central-peripheral gradient. The contribution of growth (eG) increased ($r = 0.56$, $p = 0.04$, Fig. 8a), while that of survival (eL) decreased with centrality ($r = 0.57$, $p = 0.03$, Fig. 8b).

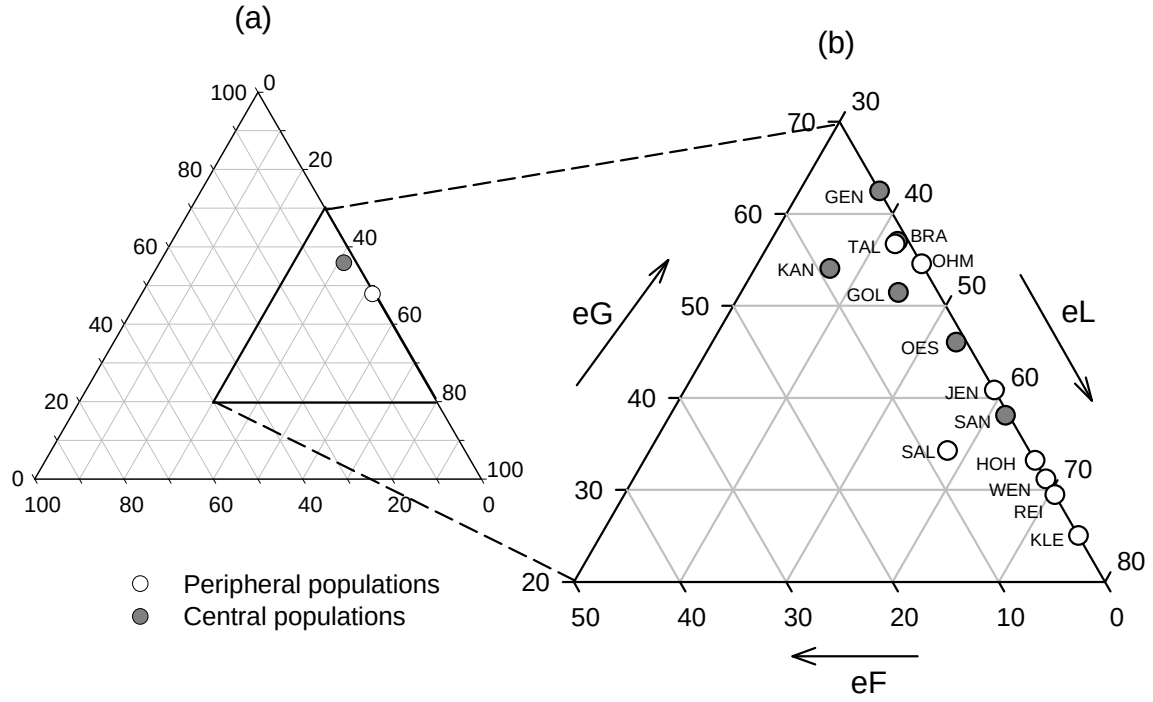

Figure 7. Triangular G-L-F ordination of the elasticities of the mean matrices (2008-2011) for peripheral and central populations of *Carduus defloratus*. (a) Means for the two types, (b) individual populations. For abbreviations of population names see Table 1.

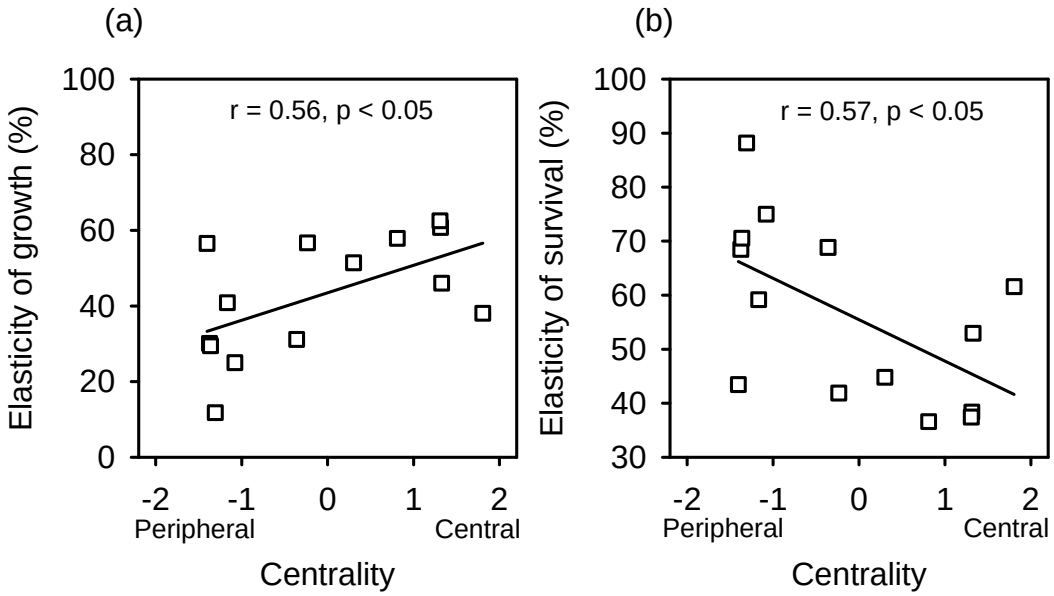

Figure 8. Relationships between the regional elasticities of (a) growth (eG) and (b) survival (eL) and centrality for 14 populations of *Carduus defloratus* in Central Europe.

Life table response experiment analysis

We used factorial LTRE analysis to study the effect of population type, transition interval and their interaction on population growth rate of *C. defloratus* and to determine the contribution of individual transitions to these effects. The LTRE analysis indicated that although the overall mean growth rates of peripheral and central populations were very similar, there were strong differences in the contributions of individual demographic transitions to λ, which would on their own result in differences in λ (Fig. 9b). Higher contributions of stasis of the vegetative ramets and retrogression of flowering ramets to λ in peripheral than in central populations would have resulted in higher λ there, but were compensated by the lower contributions of progression of vegetative to flowering ramets and stasis of flowering ramets. The strongest differences in transition rates (Fig. 9a) did not always have the strongest effect on differences in λ. For instance, the much lower rates of seedling recruitment and seedling survival in peripheral than in central populations (Fig. 9a) did not contribute to differences in λ (Fig. 9b).

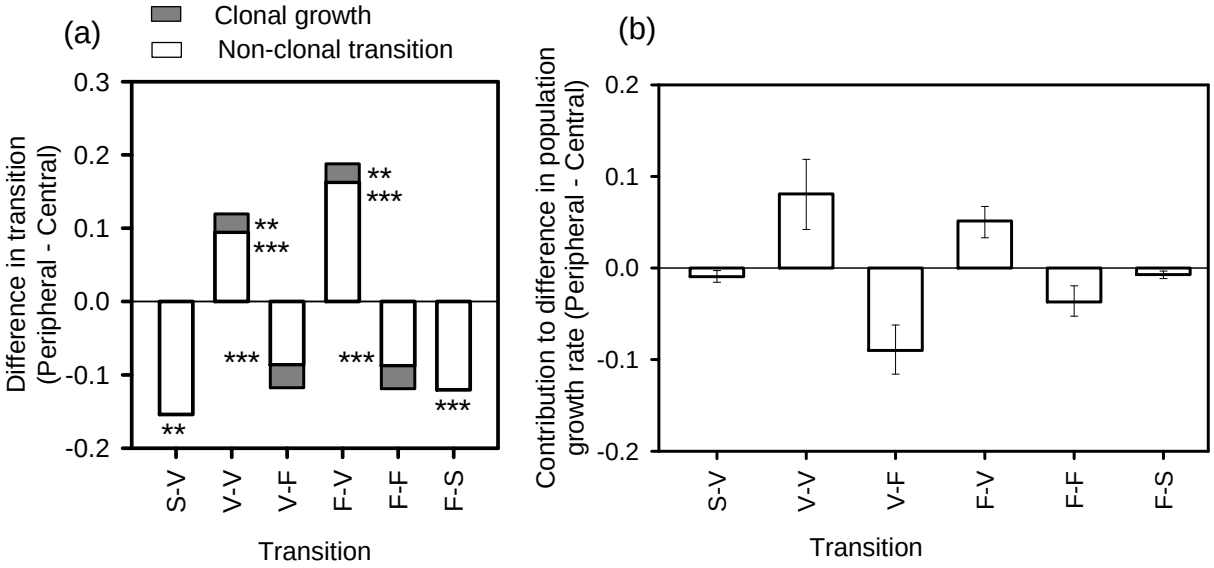

Figure 9. (a) Differences between individual demographic transitions between peripheral and central populations of *Carduus defloratus,* and (b) the contribution of these differences to the difference in population growth rate. S = seedling, V = vegetative ramet, F = flowering ramet. **, $p < 0.01$ for difference between population types; ***, $p < 0.001$. Error bars indicate 95% bootstrap confidence intervals for contributions.

The population growth rate of *C. defloratus* varied strongly among years. In 2008-09, λ was much lower than on average (λ = 0.901 vs. 0.965). This was due to lower values for all transitions involving adult ramets, which contributed to the lower growth rate (Fig. 10a, b). In 2009-10, λ was similar to the yearly average (λ = 0.937). However, individual transitions deviated from average values in both directions and influenced λ. The particularly high growth rate in 2010-11 (λ = 1.076) was mainly due to higher than average re-emergence of vegetative ramets, and retrogression of flowering to vegetative ramets instead of death.

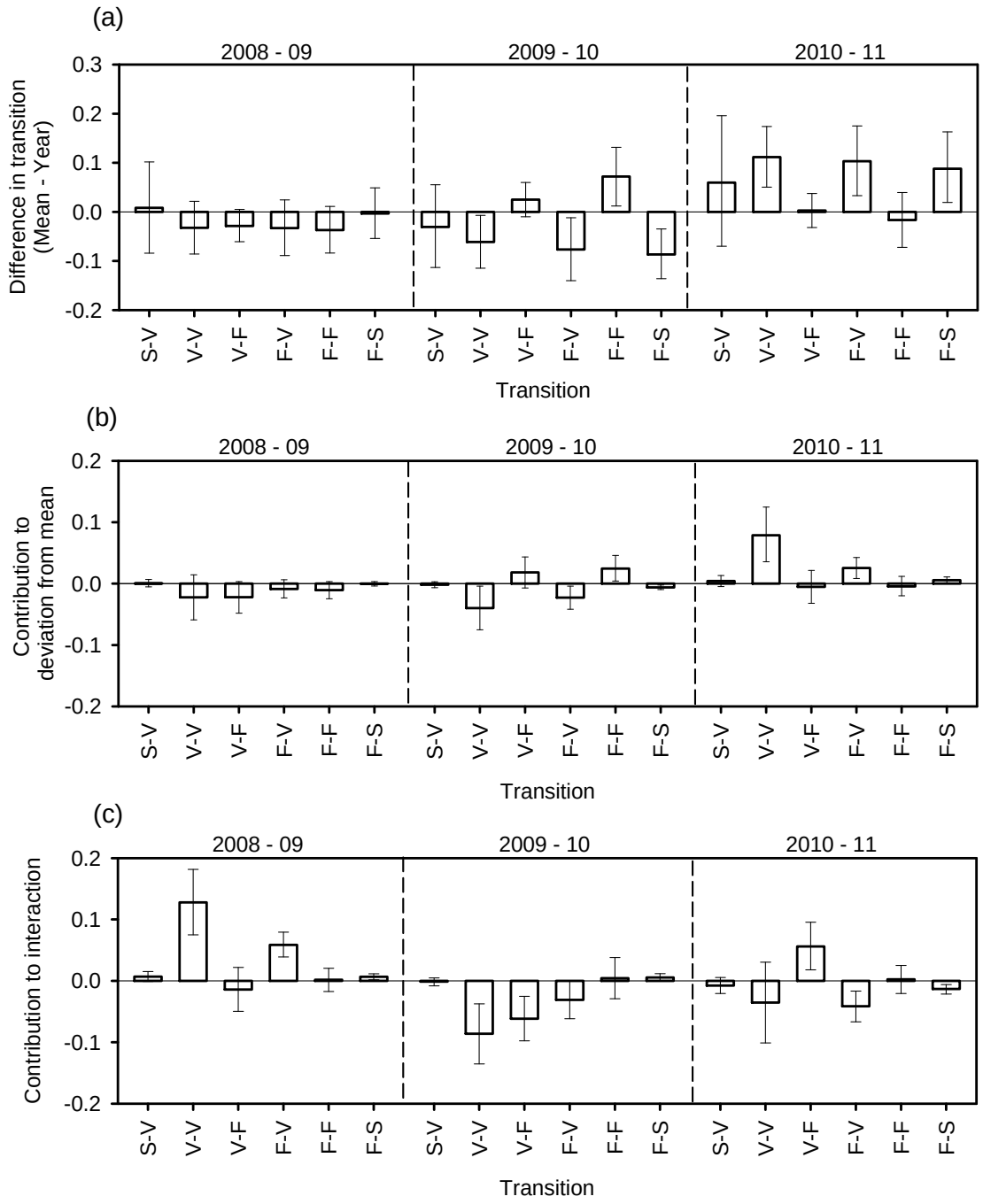

Figure 10. (a) Deviation of individual transition rates in the three transition periods from their mean over all years, and (b) the contribution of these differences to the deviation from the mean growth rate. (c) Interaction contributions, i.e. contribution of individual transitions to differences in growth rate between peripheral and central populations that could not be explained by the sum of the effects of year and population type. Results of a factorial LTRE. S = seedling, V = vegetative ramet, F = flowering ramet. Error bars indicate 95% bootstrap confidence intervals.

Population type x year interaction effects on λ, i.e. deviations from the sum of the effects of population type and transition interval, were strong (Fig. 10c). This indicates that differences in the individual demographic transitions between peripheral and central populations were not

consistent over years. The higher growth rate of peripheral than central populations in 2008-09 was due to particular high contributions of transitions (V-V and F-V transitions, Fig. 10c) that were also on average higher in peripheral populations (s. Fig. 9b). The lower λ of peripheral than central populations in 2009-10 was due to negative contributions of the same transitions, but also due to a stronger than average negative contribution of the progression of vegetative to flowering ramets (V-F). Growth rates of the two population types in 2010-11 were similar, although the contributions of several transitions (V-V,V-F, F-V) strongly differed from their average over the years (s. Fig. 9b), because these effects cancelled each other out.

Simulations based on matrix sampling indicated that overall, extinction risks for the two types of populations were similar (Fig. 11a) and that small populations consisting of less than several hundred ramets faced a relatively high risk of extinction over 100 years.

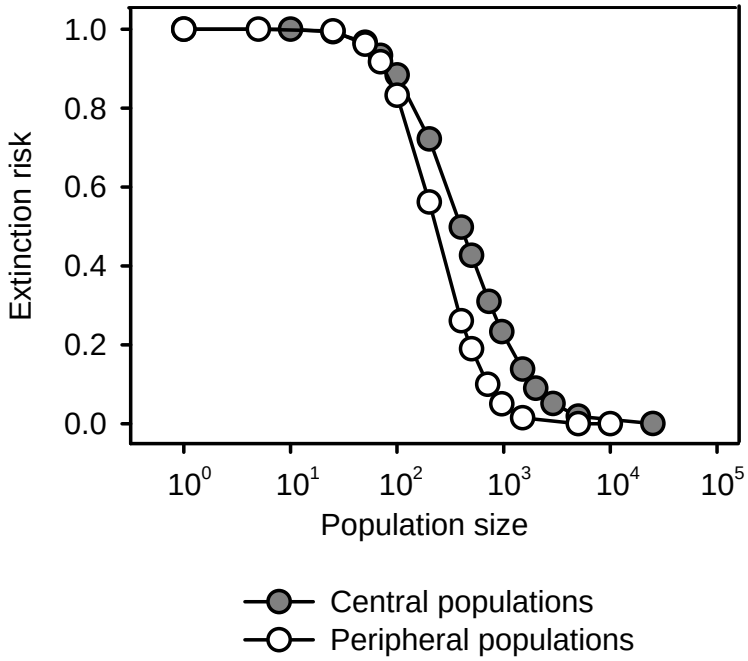

Figure 11. The relationship between population size and extinction risk over 100 years for peripheral and central populations of *Carduus defloratus*. Results of stochastic simulations of the population dynamics by matrix sampling.

DISCUSSION

In our study of the ramet demography of *C. defloratus* in 14 populations we did not find significant differences between peripheral and central populations in asymptotic growth rate

(λ), temporal variability of lambda, or the risk of extinction of populations of a given size. However, central and peripheral populations differed in individual transitions, the temporal variability of transitions, and the contribution of these transitions to population growth rates, as well as in their population structure, the speed of ontogenetic development, and the longevity of plants in different stages.

Spatial variation among populations in asymptotic growth rates (λ) was strong, but values of λ were not related to range position. Moreover, mean asymptotic and stochastic growth rates for peripheral and central populations were similar and not far from 1. This is not surprising, as longer-term deviations of λ from 1 are only to be expected if populations are strongly expanding or on their way to extinction. Long-term population studies of the orchid *Cypripedium calceolus* (García et al. 2010) found growth rates to be similar in peripheral and central populations and values close to 1. However, some other studies have reported effects of range position on λ, but their direction was not consistent. In the Californian annual *Clarkia xantiana* growth rates declined towards the range periphery (Eckhart et al. 2011), and λ was lower in a rear edge population of *Silene ciliata* in central Spain than in central populations. In contrast, growth rates were highest in British peripheral populations of two species of *Ulex* (Stokes et al. 2004). In a study of two *Mimulus* species, growth rates were highest at the range centre and lower at the periphery for *M. lewisii*, while for *M. cardinalis* the opposite was true (Angert 2006).

The temporal variability of λ in *C. defloratus* was high in comparison to that of other long-lived species (Berg 2002, Giménez-Benavides et al. 2011, Nantel & Gagnon 1999, Schleuning et al. 2008, Weppler et al. 2006, but see Schulze et al. 2012), but was not related to range position. However, variability in some individual demographic transitions like seedling survival and stasis of vegetative ramets decreased towards the range periphery. The results of the very few previous studies on plants that have compared the demography of central and peripheral populations are conflicting. In a study of two clonal perennials, Nantel & Gagnon (1999) found greater variability in some vital rates and λ in peripheral populations. Similarly, variation in several demographic rates, increased with distance to the range centre in a study of annual plants in Arizona (Gerst et al. 2011). In contrast, the temporal variation in transitions was lower in peripheral populations of *Hornungia petraea* (Kluth & Bruelheide 2005a). These conflicting patterns in the variability of λ or individual vital rates could be due to different mechanisms responsible for variation among years. Mostly, it has been predicted that variation in demography will increase towards the range edge due to greater variability in the suitability of environmental conditions (Gaston 1990, Nantel & Gagnon 1999, Gaston

2009). Alternatively, it may be the central populations that show greater variability in demographic rates, if maximum rates are higher because of very good conditions in some years (Williams et al. 2003). The increase in the variability of seedling survival and the stasis of vegetative ramets of *C. defloratus* from the periphery towards the centre supports the second hypothesis.

Temporal variation in population growth rates of plants have frequently been related to variation in weather conditions (Bengtsson 1993, Carey et al. 1995, Jump & Woodward 2003). These should affect populations in the same region in a similar way. However, while growth rates of some population of *C. defloratus* in close geographic proximity fluctuated in synchrony, others did not, indicating that differences in weather conditions alone could not explain the variation in lambda. Variation in λ could also be due to habitat management measures. Some of the central populations of *C. defloratus* were occasionally mown, resulting in increased seedling recruitment the year after and also in a higher number of ramets. However, peripheral populations were never managed and differences in management could thus not be responsible for the observed temporal variation in demography. Other possible influences on λ include site specific conditions and their interaction with weather conditions (e.g. dry periods) and biotic influences (e.g. herbivory, parasitism).

Our results provide no support for the prediction that peripheral populations of a given size have a higher extinction risk than central populations (Holt & Keitt 2000). Nevertheless, the actual extinction risk for the extant peripheral populations of *C. defloratus* is likely to be higher, because they are on average smaller than those in the distribution centre (see Chapter 2). Stochastic simulations based on mean matrices and data from four years indicated that small populations of less than several hundred individuals may be threatened in the medium term, both in the centre and the periphery. However, the four year observation period may not have been sufficiently long to obtain a representative picture of temporal variability.

Demographic transitions

The demography of *C. defloratus* was characterised by low recruitment and survival of seedlings, which is typical for long-lived species (Warner & Chesson 1985, Forbis & Doak 2004, Weppler et al. 2006). However, both elasticity analysis and LTRE analysis showed that the influence of demographic transitions involving seedlings on population growth was rather small. Ramet survival was much higher than seedling survival, but low in comparison to that

found for other species, leading to life expectancies for the ramets (< 2.5 years) that were much lower than in other clonal plants (Tanner 2001).

Most demographic transitions in *C. defloratus* were influenced by the position of a population within the distributional range. Progression of vegetative to flowering and stasis of flowering ramets increased, while retrogression of flowering to vegetative and stasis of vegetative ramets decreased with centrality. All these differences contributed to higher flowering at the range centre, where the mean proportion of plants flowering was higher. Together with the increased seed production per flowerhead (Chapter 2) this indicates higher reproduction in central populations. The gradient of increasing centrality in *C. defloratus* is at the same time a gradient of decreasing latitude, increasing altitude and a gradient in climatic conditions. Changes in climatic conditions with increasing centrality reflect much more strongly the higher altitude than the lower latitude, as temperature decreases and precipitation increases strongly (see Chapter 2). The increased flowering and reproduction in central populations of *C. defloratus* situated at higher altitudes is in line with the predictions of the abundant centre model, but in contrast to the findings of other studies that found a decrease in reproductive allocation with altitude (Young et al. 2002; Garcia & Zamora 2003, Johnston & Pickering 2004, Sakai et al. 2006, Milla et al. 2009). The contrasting pattern found in *C. defloratus* could be related to the much stronger gradient in altitude in the current than in most other studies, and the fact that *C. defloratus* is mainly an alpine species.

Like reproduction, seedling survival increased with centrality which could be related to the higher soil moisture at higher altitudes (Forbis 2003). Although the influence of reproduction and recruitment on population growth was small, the lower sexual reproduction in peripheral populations could have important long-term consequences. Lower regeneration through seeds in peripheral populations could result in reduced genetic diversity and restrict their evolutionary potential (Dorken & Eckert 2001). The peripheral populations at low altitudes represent a receding edge in the face of climate change (Hampe & Petit 2005) and a lack of sexual reproduction will reduce the opportunities for adaptive selection (Giménez-Benavides et al. 2011).

Patterns of performance along a gradient from the centre to the range limit may be complicated by the effects of density. Density dependence of performance can result in reduced mean performance where conditions are most suitable (Samis & Eckert 2007). In *C. defloratus*, there was evidence for negative density effects on plant size (Chapter 2). However, we found no negative effects of intraspecific competition on demographic

transitions in the current study. Instead, independent of centrality, seedling survival increased with density in a population, indicating that where conditions were best overall, seedling survival was highest.

Elasticity analyses indicated that the transition that contributed most to population growth was stasis of vegetative ramets (including clonal growth), whereas fecundity contributed little. Populations of *C. defloratus* are thus placed in the right hand part of the G-L-F triangle. Such a predominance of the fate of adult plants for overall demography is typical for long-lived plants (Forbis & Doak 2004, Colling & Matthies 2006, Csergö et al. 2011). However, central and peripheral populations differed strongly in the importance of different processes for population growth. While the elasticity of growth transitions (progression to the next stage) increased with the centrality of a population, elasticity of survival transitions (stasis, retrogression) decreased. The importance of different life cycle transitions and thus the position of species or populations in the G-L-F triangle have been related to secondary succession with the highest values of survival elasticities predicted for late successional species (Silvertown et al. 1993, Silvertown et al. 1996, Franco & Silvertown 2004). The pattern observed in *C. defloratus* does not fit into this framework, as peripheral populations that had the highest survival elasticities mostly inhabit sites like rocks and screes that represent early stages of primary and not late stages of secondary succession. However, a similar demographic behaviour as in peripheral populations of *C. defloratus* has been found in exclusively alpine species during the early stages of primary succession (Morris & Doak 1998, Marcante et al. 2009).

The factorial LTRE analysis of the effects of population type and transition interval showed that several of the differences in transition rates between central and peripheral populations would on their own have resulted in considerable differences in growth rate, if they had not compensated each other. The LTRE analysis thus confirmed that similar overall growth rates of populations may mask strong differences in the contribution of individual transitions to population growth (Angert 2006). Transitions found to be important in the LTRE were generally the same as identified in the elasticity analysis, indicating that retrospective and prospective analyses came to similar results. This is not always the case, as LTRE analyses describe contributions of transitions to real, observed differences in lambda, whereas elasticities indicate the projected response of lambda to very small changes in individual transitions (De Kroon et al. 2000, Caswell 2001).

CONCLUSIONS

Our results do not support the notion of greater temporal variability of demographic transitions (Lawtong 1993, Nantel & Gagnon 1999) or of greater demographic turnover (Lönn & Prentice 2002) in peripheral populations. However, detailed demographic analyses showed that peripheral differed from central populations in many features of their demography, in particular transitions involving flowering plants and the recruitment of plants from seeds. Many of the peripheral populations of *C. defloratus* may be characterised by remnant population dynamics (Eriksson 1996), as no recruitment was observed in them during four years of study. The existing populations persist due to clonal growth, but new populations are not formed due to a lack of suitable habitats and the very limited dispersal of seeds.

A main result of the current study is the strong spatial and temporal variation in demography. Recently, Jongejans et al. (2010) have criticised that hardly any studies on plant demography have investigated populations at more than one site in different regions. In their study of three species of Asteraceae they found variation in life histories over the distribution range of the species. The strong variation in individual demographic transitions and their elasticities found among populations of *C. defloratus* in different parts of its range supports the view that it is not sufficient to study the demography of a species at only one site or even region to characterise its population dynamics (Jongejans et al. 2010). However, the fact that demographic features of *C. defloratus* showed clinal variation related to gradients in centrality and thus climate, suggest that it might be possible to predict general demographic features for individual populations based on their environment.

CHAPTER 4

Genetic patterns of an alpine plant across its Central European distribution are in line with the predictions of the abundant centre model

in preparation
with Diethart Matthies

ABSTRACT

The abundant centre model (ACM) predicts that genetic diversity within populations should decrease towards the range limits, while the genetic differentiation between populations should increase. To test the genetic predictions of the ACM, we analysed patterns of genetic variation of *Carduus defloratus* across 78 populations in Central Europe using AFLPs. The populations were distributed along a central-peripheral gradient from the Alps towards the northern range limit of the species in Germany, where the species is considered to be a glacial relict. In line with the predictions of the ACM, genetic diversity within populations decreased and genetic differentiation between populations increased with latitude towards the range margin. Genetic diversity also increased with population size, but population size did not explain a significant part of the variation in addition to range position, suggesting that present-day genetic patterns of the species are mainly affected by historical processes. Strong isolation by distance patterns among peripheral populations in spite of their current isolation indicated that the genetic structure of the long-lived species at the range periphery may reflect patterns of gene flow during the last ice age when populations were probably much more common. Genetic differentiation increased much more strongly with geographic distance for peripheral than for more central populations, indicating stronger isolation.

INTRODUCTION

The genetic structure of populations depends on life history traits and on ecological factors influencing reproduction and dispersal (Hamrick & Godt 1996). Ecological factors can also affect population genetic processes like genetic drift, gene flow, bottlenecks, and selection which in turn influence the genetic structure of natural populations (Hutchison & Templeton 1999). Founder effects, genetic drift, and bottlenecks reduce the genetic variability within populations and result in greater genetic differentiation between populations. In plant species founder effects may be mitigated by gene flow due to seed and pollen dispersal which increase genetic variability and reduce differentiation between populations (Hutchison & Templeton 1999, Vik et al. 2010). The effects of selection differ, depending on whether selection is convergent or divergent (Endler 1982, Volis et al. 2005). Convergent selection favours the same genotypes at different sites, resulting in low population genetic structure. In contrast, divergent selection favours different genotypes at different sites, resulting in strong differentiation among populations.

The relative importance of genetic drift, gene flow, and selection for genetic variability and structure may be affected by the position of populations within the distributional range of a species (Brown et al. 1996). The range position can therefore have important implications for the evolutionary potential of populations and their conservation value (Hoffman & Blows 1994, Lesica & Allendorf 1995). The Abundance Centre Model (ACM; Brown 1984) predicts that due to decreasing favourability of conditions from a distribution centre of a species towards its range limit, populations should become less frequent, less dense, smaller, and less reproductive (Brown 1984, Lawton 1993, Sagarin & Gaines 2002, Jump & Woodward 2003). As a consequence of the smaller size of populations and their greater isolation, the relative influence of genetic drift on the genetic structure of populations should increase towards the range margin (Ellstrand & Elam, 1993, Lesica & Allendorf, 1995). Therefore, genetic diversity within populations should decrease, whereas genetic differentiation among populations should increase towards the range limit. This pattern may be further strengthened if peripheral populations are more strongly affected by inbreeding or severe population bottlenecks, and extinction and recolonisation events are more frequent than in central populations (Lesica & Allendorf 1995, Eckert et al. 2008).

However, reviews of empirical studies of various groups of organisms concluded that the predictions of the ACM regarding patterns of abundance were often not supported (Sagarin & Gaines 2002, Sexton et al. 2009), which should also reduce the support for the predicted genetic patterns. Moreover, even if patterns of abundance follow an abundant centre

distribution, other factors may have strong effects on the pattern of genetic diversity. Longevity, obligate outcrossing, and long distance dispersal may reduce the predicted effects of small population size and geographical isolation (Loveless & Hamrick 1984; Schiemann et al. 2000).

A recent review of genetic diversity and structure across geographical ranges of various groups of organisms concluded that most of the available studies detected the expected decline in neutral genetic diversity towards the range margin and also found increased differentiation, but that this supposition was still poorly tested (Eckert et al. 2008). In only 24 out of 81 studies both predictions had been subjected to statistical tests. It has also been criticised that many studies included only a small number of peripheral populations or compared a few peripheral populations to central ones which gives little information about the form of a possible relationship between genetic structure of populations and their range position (Sagarin & Gaines 2002, Samis & Eckert 2007, Eckert et al. 2008, Yakimowski & Eckert 2008, Sexton et al. 2009). Moreover, often genetic distances between populations increase with their geographic distance (isolation by distance, Hutchison & Templeton 1999). In this case, the mean genetic distance will depend on the geographic distance between the populations sampled, and this has to be taken into account when comparing the genetic differentiation between populations in different groups, but has rarely been done (Nybom 2004, Hamilton & Eckert 2007).

The variation in population genetic diversity and differentiation across the entire range of a species will be affected by both historical and contemporary effective population sizes and gene flow (Vucetich & Waite 2003, Hamilton & Eckert 2007). Historical factors are those that influenced the population genetic structure of a species in the past and do not affect it in the present, but the genetic pattern influenced by them may still be reflected in contemporary populations (Vucetich & Waite 2003, Yakimowski &Eckert 2008). Examples for historical factors are range fragmentations due to glaciation and subsequent founder events during postglacial range expansion (Schönswetter et al. 2003, Meeus et al. 2012), or habitat fragmentation due to human activities in past centuries (Gibbs 2001, Storfer et al. 2007, Zhang et al. 2012). In contrast, contemporary factors presently influence the distribution of genetic diversity. These factors include population size, and features of the demography of populations that influence effective population size or obstruct current gene flow (Yakimoswski & Eckert 2008). However, most of the studies (81%) that have compared the genetic structure of peripheral and central populations did not take population size into account (Eckert et al. 2008) and few have tried to distinguish between possible historical and

contemporary effects on population genetic diversity and structure (Lönn & Prentice 2002, Vucetich & Waite 2003, Hamilton & Eckert 2007, Meeus et al. 2012).

We studied the genetic diversity of 78 populations of *Carduus defloratus* L. (Asteraceae) and the differentiation between them along a gradient from the centre of its distribution in the Alps to its northern range limit in the central German low mountain ranges. The pattern of abundance of *C. defloratus* is in agreement with the predictions of the ACM: population size, plant density, and seed set decrease from the centre of its distribution towards the northern range limit (Chapter 2). We estimated the genetic diversity within populations and genetic differentiation between populations using AFLP markers and related genetic diversity and genetic distinctness of populations to their latitude, longitude, and size. We also investigated the relationship between the genetic differentiation and the geographical distance between populations. We address the following specific questions: (1) Does genetic diversity decrease from the distribution centre in the Alps towards the northern range limit and is it affected by population size? (2) Is genetic differentiation between populations higher at the northern range limit than further south and does the mean genetic distinctness of populations increase towards the northern range limit? (3) Does the genetic distance between populations increase more strongly with geographic distance for populations at the northern range limit than for more central ones?

MATERIAL AND METHODS

Study species

C. defloratus is a European species with a large altitudinal range (300 - 2600 m; Landolt 2003). It occurs in central Europe from the Alps to the central German low mountain ranges (Fig. 1). It also occurs south of the Alps in the Italian Appenines, further southwest in the Pyrenees and further east in the Tatra mountains (Meusel & Jäger 1992). In the Alps *C. defloratus* is a common species of alpine grassland and on rocks at higher altitudes, where it covers large, continuous areas. In contrast, towards the northern range limit the species becomes increasingly rare and is restricted to rocks, cliffs and open woodlands where trees are scarce, but partially shading the populations.

C. defloratus is a diploid, long-lived clonal perennial with a mixed mating system. The achenes possess a pappus and are dispersed by wind, but are large (2 mg). Dispersal is thus likely to be very limited, as in the related *C. nutans* and *C. acanthoides* for which dispersal of less than 5 m has been found (Skapaas & Shea 2007). That makes gene flow via seed

dispersal between the very disjunct populations at the northern range limit very unlikely as the median nearest neighbour distance between them is more than 6 km. *C. defloratus* is not yet considered to be threatened in Central Europe, but it is included in the advance warning list of the red list of German federal states of Hesse (Buttler et al. 1997) and Baden-Württemberg (Breunig & Demuth 1999) due to its rarity.

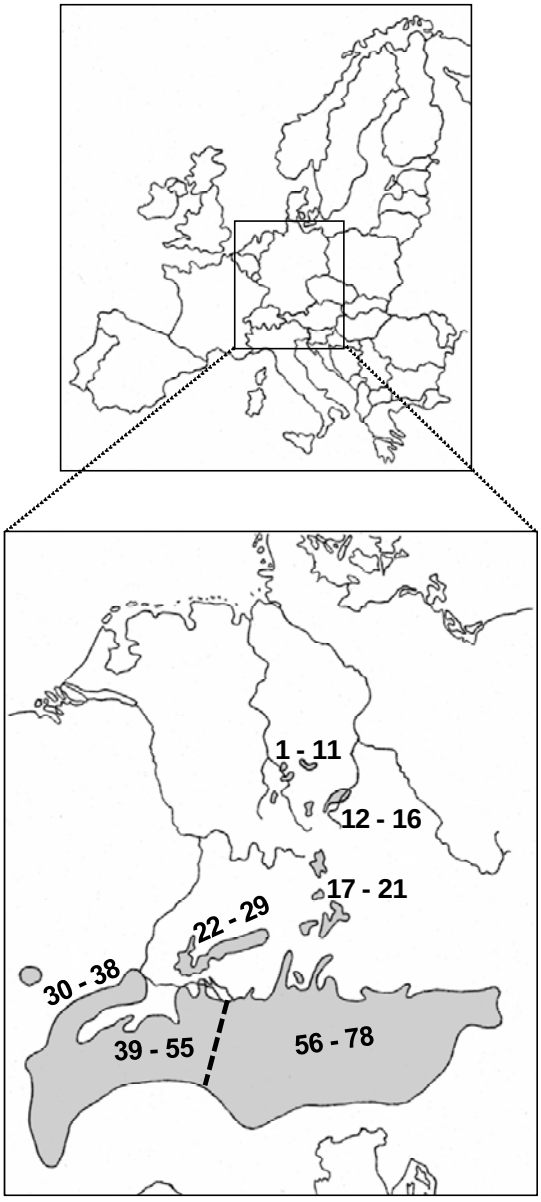

Figure 1. Map of the distribution of *Carduus defloratus* ssp. *defloratus* in Central Europe (after Meusel & Jäger 1992). The numbers indicate studied populations within seven mountain regions. Population 1 - 11 Werrabergland and Ohmgebirge; 12 - 16 Ilm-Saale-Platte; 17 - 21 Franconian Alb; 22 - 29 Swabian Alb; 30 - 38 Swiss Jura; 39 - 55 W-Alps; 56 - 78 E-Alps.

Collection of samples

In summer 2006, 78 populations of *C. defloratus* were studied across the species' range in Central Europe, from the centre of the distribution in the Alps towards the northern range limit in the low mountain ranges of central Germany (Fig. 1). Because of the topographic profile of Central Europe the north-south gradient was simultaneously an elevation gradient. The altitudinal range of the populations sampled was 342 - 2300 m, the maximum distance 679 km. We defined a population as a group of conspecific individuals that were at least 1 km apart from the next group or if not, were clearly separated by woodland, valleys, or agricultural fields. We sampled populations in seven mountain regions: From different altitudes in (1) the W-Alps, (2) the E-Alps, and (3) the Swiss Jura mountains. However, populations at the upper altitudinal limit in the Alps were difficult to locate and therefore not sampled. We also sampled from populations in (4) the Swabian Alb, (5) the Franconian Alb, (6) the Ilm-Saale Platte, and (7) the Ohmgebirge and Werrabergland. The populations in the Alps can be clearly considered as central, while those at the northern distribution limit (regions 6 and 7) can be considered peripheral. At the northern range limit all accessible populations were sampled.

The longitude, latitude, and altitude of each population were determined with a GPS. Population size was determined as the number of flowering plants. In each population one fresh leaf was sampled from 10 to 30 plants and stored in silica gel until the extraction of DNA. In small populations (< 30 individuals), all accessible plants were sampled. To reduce the chance of sampling clones, the minimum distance between two sampled plants was 0.5 m.

DNA extraction and AFLP analysis

The dried leaf material (20 mg, without midnerve and prickles) was ground (Retsch MM200, Retsch). Subsequently, DNA was extracted using the DNeasy Plant Mini Kit (Qiagen). For the molecular genetic analysis dominant amplified fragment length polymorphism (AFLP) markers were used (Vos et al. 1995). Restriction and ligation were carried out by the AFLP CoreReagent Kit (Invitrogen). For preselective amplification AFLP Pre-Amp Primer Mix I (Invitrogen) and Taq polymerase were used that contained BSA (5 u μL^{-1}, native, with BSA, Fermentas). Three primer combinations were used for selective amplification: FAM-ACA/CTC, TAM-AAC/CAT and HEX-ACG/CTT (Invitrogen). The PCR amplification products were separated by capillary electrophoresis using an automated DNA sequencer (MegaBACE 500, 48-capillary system, GE Healthcare).

DATA ANALYSIS

Allele frequencies and genetic diversity

The presence or absence of each marker in each individual was scored by the MegaBACE Fragment Profiler version 1.2. Only fragments ranging in length between 150 and 500 base pairs were included in the analysis. The resulting presence/absence matrix included 435 polymorphic loci. The error rate was estimated at 4.8% by duplicate analysis of 30 samples.

We counted the number of private alleles within each population. Allelic frequencies were estimated by both a square-root procedure (Lynch & Milligan 1994, Stewart & Excoffier 1996) and a Bayesian method assuming non-uniform prior distribution of allele frequencies (Zhivotovsky 1999). Because nothing was known about the selfing rate of *C. defloratus* we assumed random mating and that the populations were in Hardy-Weinberg equilibrium (i.e. $F_{IS} = 0$) in all statistical analyses. First we calculated the square root of the frequency of null homozygotes (frequency of band absences) and used that to calculate the frequency of null alleles (Stewart & Excoffier 1996). To take into account the different numbers of individuals analysed per population, we rarefied the data prior to the estimations by randomly sampling 10 individuals (1000 simulations). In one population with only five individuals all individuals were analysed. Two estimates of within population genetic diversity were calculated: the percentage of polymorphic loci across loci and the expected Nei‘s gene diversity (H_e, Lynch & Milligan 1994, Peakall & Smouse 2005). For the alternative Bayesian allele frequency estimation procedure of Zhivotovsky (1999) we used the programme AFLP-SURV V1.0 (Vekemans et al. 2002). All measures of genetic diversity were strongly correlated ($r = 0.71 – 0.96$, $p < 0.01$), and we therefore present in the following only the results of the square-root, but rarefied estimate (H_e). Differences among mountain regions in genetic diversity were studied by analysis of variance. We investigated the relationship between genetic diversity and the explanatory variables longitude, latitude and population size by multiple regression analysis. Regressions were calculated with all possible combinations of explanatory variables. The Bayesian information criterion (BIC) was used to measure the strength of evidence for each tested model (Schwarz 1978). The models with the lowest BIC are presented in the results. Relationships between variables were illustrated by partial regression plots. Partial regression plots adequately present the results of multiple regression analyses as they show the effect of an explanatory variable after removing the effects of the other predictors (Moya-Laraño & Corcobado 2008).

Genetic differentiation

Based on allelic frequencies estimated by a square-root procedure, we calculated a matrix of pairwise Phi_{PT} values as implemented in GenAlEX 6.41 (Peakall & Smouse 2005). In a second approach we calculated a matrix of pairwise F_{ST} values on the basis of allelic frequencies estimated by the Bayesian method implemented in AFLP-SURV V1.0 (Vekemans et al. 2002). The matrices of pairwise Phi_{PT} and F_{ST} values were highly correlated ($r_M = 0.95$, $p < 0.001$, 1000 permutations, Mantel 1967), and therefore in the following only the results of the pairwise Phi_{PT} values are reported.

To visualise possible patterns of genetic differentiation between populations we performed a Principle Coordinate Analysis (PCoA) with GenAlEx 6.41 based on pairwise Phi_{PT} values. Separation of the northern peripheral from the other populations along the first two PCoA axes was evaluated by ANOVAs. To quantify the distribution of genetic variation at different hierarchical levels we used analysis of molecular variance (AMOVA). We first calculated a four-level AMOVA using the total data set to study the partitioning of molecular variance (1) between peripheral northern and the other populations, (2) among the seven mountain regions within population types, (3) among populations within mountain regions, and (4) among individuals within populations. For a more focused test of the predictions of the abundant centre model, we reduced our data set and compared the peripheral northern with the central populations in the Alps with a three-level AMOVA (n = 56), and also compared the partitioning of molecular variance among and within populations for each population type separately. Analyses were carried out using the *varcomp.glob* function of the *hierfstat* R-package (Goudet 2005), and the significance of variance components was tested using 1000 permutations with the *test.within* and *test.between.within* functions of the package.

Following Yakimowski & Eckert (2008) we tested the prediction that population genetic divergence should increase towards the range limit and with decreasing population size by calculating the mean pairwise genetic distance of each population from all others (mean Phi_{PT}) and related it to latitude, longitude and population size. We tested the significance of the regressions by permuting the matrix of pairwise Phi_{PT}-values 10 000 times and calculating the probability of obtaining an r^2-value higher than that observed (two-sided test).

Large and small scale population structure

To test for isolation by distance patterns in the genetic structure, we performed correlations between pairwise genetic and geographic distances and evaluated them using Mantel tests

with 999 permutations (GenAlEx 6.1). We used the matrices of pairwise linearised Phi_{PT} values [$linPhi_{PT} = Phi_{PT} / (1 - Phi_{PT})$; Rousset 1997] and log-transformed geographical distances. Analyses were performed using (1) all populations together, (2) separately for the peripheral northern populations and the other populations. To analyse whether mean pairwise $linPhi_{PT}$ values differed between various groupings of populations corrected for pairwise geographic distance, and whether there were differences in regression slopes, we calculated general linear models and evaluated the significance of terms with permutation tests (10 000 permutations), using an R-script (R 2.12.1, R Development Core Team 2011). The reported p-values were derived from the proportion of times the estimated F-values exceeded the observed values. Analyses were performed for (1) all populations to study differences between the peripheral northern and all other populations, and (2) the peripheral northern populations and those from the Alps. In all these analyses only distances within each group were used.

To study the genetic population structure at a smaller scale, we evaluated the relationship between pairwise linearised genetic and geographic distances between pairs of populations that were not more than 11 km from each other. These analyses were carried out separately for all populations, and for the peripheral northern populations and those from the Alps.

Bayesian population assignment

To test how individuals are assigned to regions without prior information about their origin we carried out a Bayesian cluster analysis (STRUCTURE 2.3.1, Pritchard et al. 2000, Pritchard & Wen 2004). We assumed that populations were not admixed and that allele frequencies were correlated. The length of the burn-in and Markov-chain Monte Carlo (MCMC) was set to 10000; longer burn-in or MCMC did not change our results significantly. To find the optimum number of clusters (K) we analysed a series of 30 independent runs for each predefined *K* ranging from 1 to 16 and inspected the mean values of L(K), $L'(K)$, $L''(K)$ and ΔK, as recommended by Evanno et al. (2005). To align the runs of the STRUCTURE simulation we used the programme CLUMPP V1.1.2 (Jakobsson & Rosenberg 2007).

For each individual we calculated the provability of assignment to a certain cluster (Q). Subsequently, we calculated the mean Q of an individual within a population. To test whether mean Q was influenced by population type or mountain region we performed ANOVAs. We also evaluated the relative influences of population size, longitude, and latitude on mean Q by

multiple regression analysis. We present the results of models with the lowest BIC and illustrate the relationships between variables by partial regression plots.

RESULTS

Allele frequencies and genetic diversity

Mean genetic diversity within populations (H_e) was 0.054 and differed significantly among mountain regions ($F_{6,77} = 6.46$, $p < 0.001$). Populations in the Alps were on average most genetically diverse, while those at the northern range limit were least diverse (Table 1). Simple regressions indicated that the genetic diversity of a population increased with its size ($r = 0.29$, $p = 0.010$) and with altitude ($r = 0.45$, $p < 0.001$), but in the model with most support according to the minimum BIC only latitude and longitude were included. The genetic diversity of a population strongly decreased with latitude from the centre of the distribution in the south towards the northern range limit (Table 2, Fig. 2a), and increased less strongly from west to east (Fig. 2b).

Table 1. Mean gene diversity ($H_e \pm 1$ SE) in populations of *Carduus defloratus* in seven mountain regions. Genetic diversity within populations differed significantly among regions ($F_{6,77} = 6.46$, $p < 0.001$).

Region	H_e	Number of studied populations
Ohmgebirge & Werrabergland	0.045 ± 0.002	11
Ilm-Saale-Platte	0.046 ± 0.002	5
Franconian Alb	0.053 ± 0.005	5
Swabian Alb	0.051 ± 0.002	8
Swiss Jura	0.054 ± 0.002	9
W-Alps	0.055 ± 0.001	17
E-Alps	0.059 ± 0.002	23

Table 2. Significant influences on mean gene diversity (H_e) in 78 populations of *Carduus defloratus* in Central Europe. The model is presented for which the Bayesian information criterion is minimal. Potential explanatory variables were latitude, longitude, and population size.

Dependent variable	r^2	F	Explanatory variable	β	t
H_e	0.41	26.22***	Latitude	-0.62	-6.81***
			Longitude	0.38	4.10***

Notes: degrees of freedom, 75; ***, $p < 0.001$.

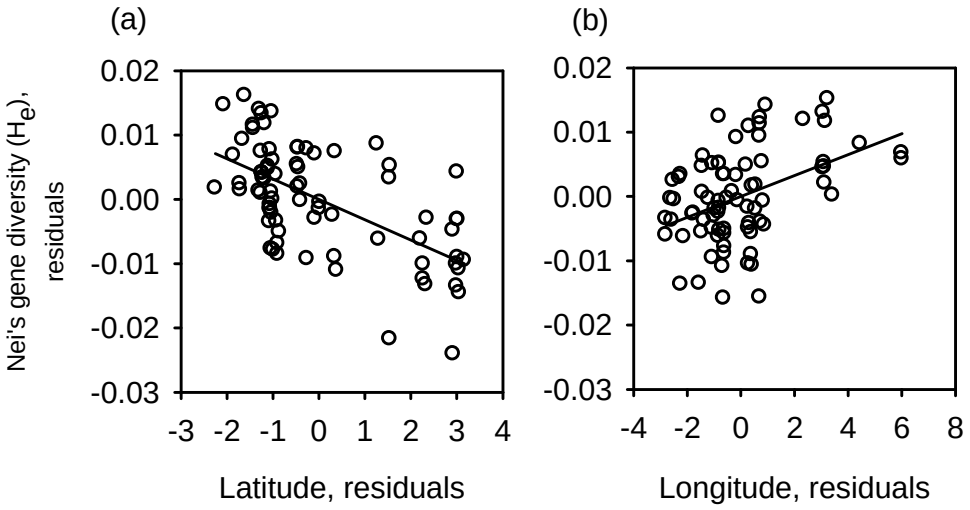

Figure 2. The relationship between the mean genetic diversity in populations of *Carduus defloratus* and (a) their latitude and (b) their longitude. Shown are partial regression plots of the influence of an explanatory variable after partialing out the effects of the other variable in the multiple regression model. For statistical analysis see Table 2.

Genetic differentiation

About 51% of the total variation in pairwise Phi_{PT} values was explained by the first two PCoA axes. The very disjunct populations at the northern range limit and the southern populations were well separated along the first PCoA axis ($F_{1,76} = 109.04$, $p < 0.001$, Fig. 3). An AMOVA also indicated that the peripheral northern populations were genetically differentiated from the rest of the studied populations, but the proportion of variation due to these differences was quite small (Table 3a). Slightly larger was the differentiation of the peripheral populations from the central populations in the Alps (Table 3b). The proportion of variation due to genetic

differentiation among populations was higher for the peripheral northern populations (16.1% of the variation, Table 3c) than for the other populations (12.1%, Table 3d).

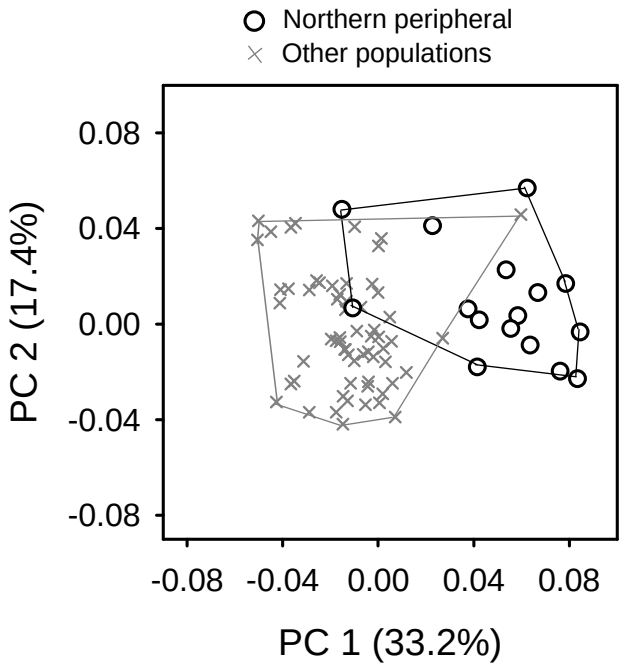

Figure 3. Two-dimensional plot of the Principle Coordinate Analysis of pairwise genetic distances (Phi_{ST}) between 78 Central European populations of *Carduus defloratus*. The northern peripheral and the other, more central populations are delimited by minimum convex polygons.

Table 3. Partitioning of genetic variation by analyses of molecular variance among variously defined groups of populations, and among and within populations of *Carduus defloratus*.

Source of variation	df	VC	Proportion (%)
(a) *All populations*			
Peripheral northern vs. other	1	1.48	3.7*
Among mountain regions within groups	5	0.76	1.9**
Among populations within mountain regions	71	4.48	11.0**
Within populations	932	33.89	83.5**
(b) *Peripheral northern and populations from the Alps*			
Peripheral northern vs. Alps	1	1.08	5.2**
Among populations within groups	54	2.39	11.7**
Within populations	669	16.97	83.0**
(c) *Peripheral northern populations*			
Among populations	15	2.64	16.1**
Within populations	197	13.69	83.9**
(d) *All except peripheral populations*			
Among populations	61	2.46	12.1**
Within populations	735	17.82	87.9**

Notes: df, degrees of freedom; VC, variance component; *, $p < 0.05$; **, $p < 0.01$.

Large and small scale spatial genetic structure

Linearised genetic distances ($linPhi_{ST}$) between pairs of populations increased significantly with their geographical distances in the total data set ($r_M = 0.35$, $p < 0.01$) and among populations of both the peripheral northern ($r_M = 0.59$, $p < 0.01$) and the other populations separately ($r_M = 0.30$, $p < 0.01$, Fig. 4a). The mean pairwise $linPhi_{PT}$ value was significantly higher (Table 4a) among the peripheral northern than among the rest of the populations (0.207 vs. 0.146,), even when differences in geographical distance were taken into account. Genetic

distance increased far more strongly with geographical distance among populations at the northern range limit than among the other populations (slope of the regression line, b, 0.062 vs. 0.037). A very similar pattern was found when the very disjunct, northern populations were compared only with the populations in the Alps (Table 4b). Population genetic divergence (mean $linPhi_{PT}$) increased strongly with latitude, i.e. towards the range limit ($r = 0.62$, $p < 0.001$, permutation test; Fig. 5), decreased with altitude ($r = - 0.38$, $p < 0.01$) and population size ($r = - 0.32$, $p < 0.01$), and was not influenced by longitude ($r = 0.18$, $p = 0.12$). In multiple regressions, none of the other variables increased significantly the r^2 of the model with only latitude as predictor (all $p > 0.22$).

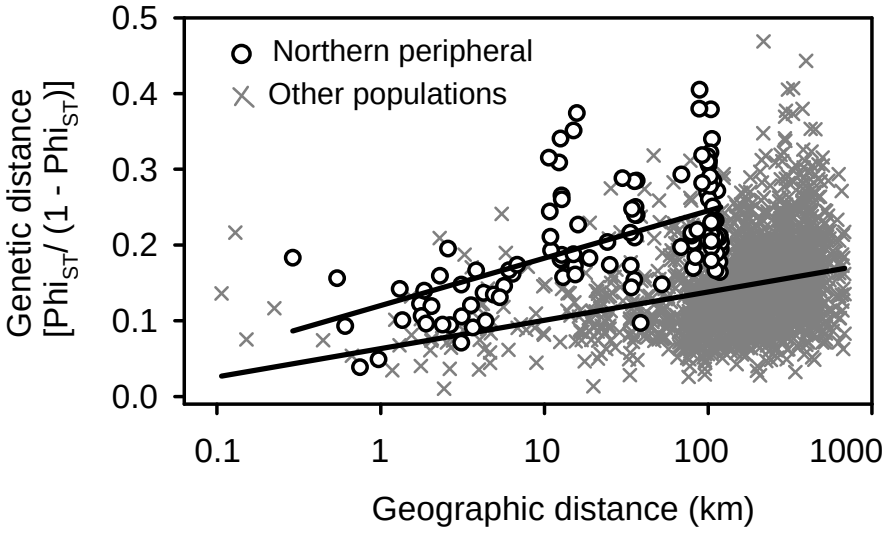

Figure 4. The relationship between linearised pairwise genetic and geographic distances between populations of *Carduus defloratus* in Central Europe. Comparison of the peripheral northern and other populations.

Table 4. Results of general linear models of the effect of geographical distance, population groupings and their interaction on linearised pairwise genetic distances between populations of *Carduus defloratus* in Central Europe at two spatial scales. (a) Among all populations, maximum distance 679 km; (b) among the peripheral northern (maximum distance 119 km) and the populations from the Alps (maximum distance 666 km). The analysis at the small spatial scale only includes pairs of populations less than 11 km apart from each other. P-values were obtained by permutation tests using 10000 random permutations.

		Large spatial scale		*Small spatial scale*	
Source of variation	df	F	p	F	p
(a) *All populations*					
Geographic distance	1	99.41	< 0.001	2.84	0.09
Type (Peripheral northern vs. other)	1	273.99	< 0.001	14.15	< 0.001
Distance x Type	1	9.98	< 0.01	8.27	< 0.01
(b) *Peripheral northern and populations from the Alps*					
Geographic distance	1	32.82	< 0.001	10.09	< 0.01
Peripheral northern vs. Alps (Type)	1	461.58	< 0.001	25.83	< 0.001
Distance x Type	1	19.25	< 0.001	2.76	0.10

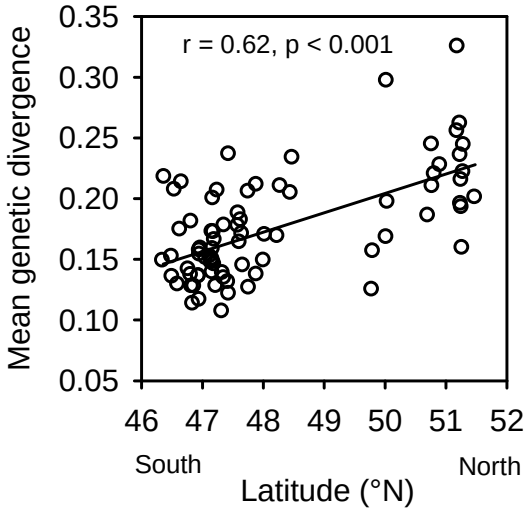

Figure 5. The relationship between population genetic divergence, measured as the mean genetic distance between a population of *Carduus defloratus* and all other populations, and latitude.

At a scale of less than 11 km, the genetic distance between pairs of populations increased significantly with geographic distance among the northern peripheral populations, but not among the other populations (slope 0.072 vs. -0.002; Fig. 6a, Table 4a), and the peripheral populations were thus on average stronger differentiated than the others ($linPhi_{ST}$, 0.138 vs. 0.099). Across the peripheral northern and the central populations in the Alps, pairwise genetic distances increased significantly with pairwise geographic distances (Table 4b). Compared with the total data set, the effect of group was increased, but differences in b were not significant (0.072 vs. 0.026).

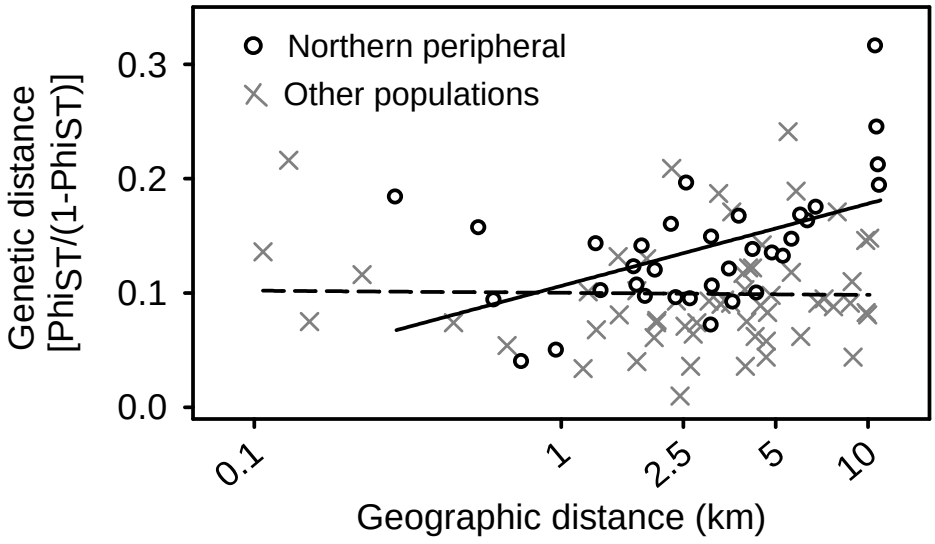

Figure 6. Small-scale population differentiation in *Carduus defloratus*. The relationship between linearised pairwise genetic and geographic distances between populations. Only distances between populations that were less than 11 km from each other were included. Solide line indicates northern peripheral populations, dashed line indicates the other populations; for statistics see text.

Bayesian population assignment

The optimum number of two clusters (K = 2) corresponded to the number of population types. However, in no population were all individuals assigned to a single cluster (Fig. 7a). Nevertheless, the mean probability of an individual to be assigned to cluster 2 was larger in the peripheral northern than in the rest of the populations (79.5% vs. 37.6%, $F_{1,76} = 35.73$, $p < 0.001$, Fig. 7b). The mean probability of assignment to cluster 2 varied significantly among the mountain regions ($F_{7,70} = 7.93$, $p < 0.001$). The regression model with the lowest BIC included the explanatory variables latitude and longitude ($r^2 = 0.40$, $F_{2,75} = 25.30$, $p < 0.001$). The mean probability of individuals of assignment to cluster 2 increased significantly with the

latitude ($\beta = 0.60$, $t = 6.46$, $p < 0.001$) of the populations, but decreased with longitude ($\beta = -0.42$, $t = -4.50$, $p < 0.001$, Fig. 8a,b).

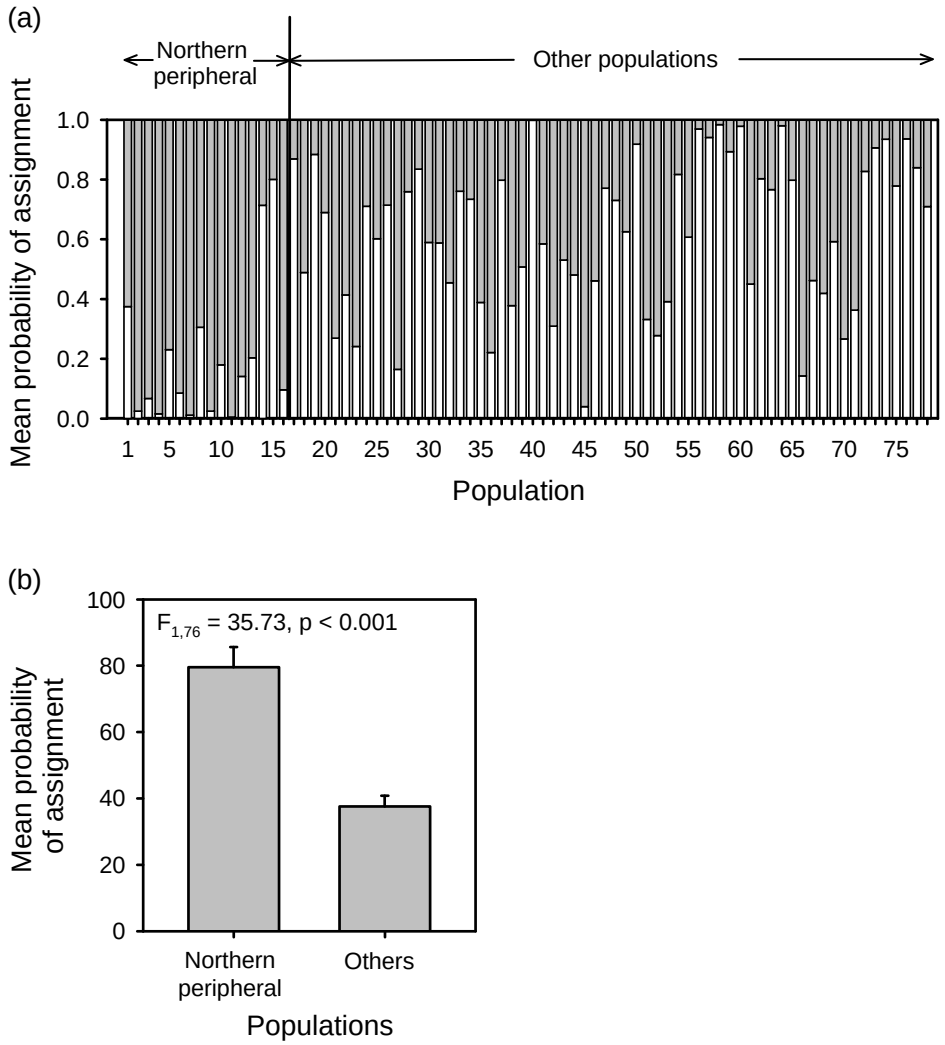

Figure 7. Results of Bayesian cluster analysis of AFLP-haplotypes in 78 populations of *Carduus defloratus*. (a) Mean probability that an individual of a population is assigned to each of two clusters (b) Mean probability of northern peripheral and other populations of being assigned to cluster 2.

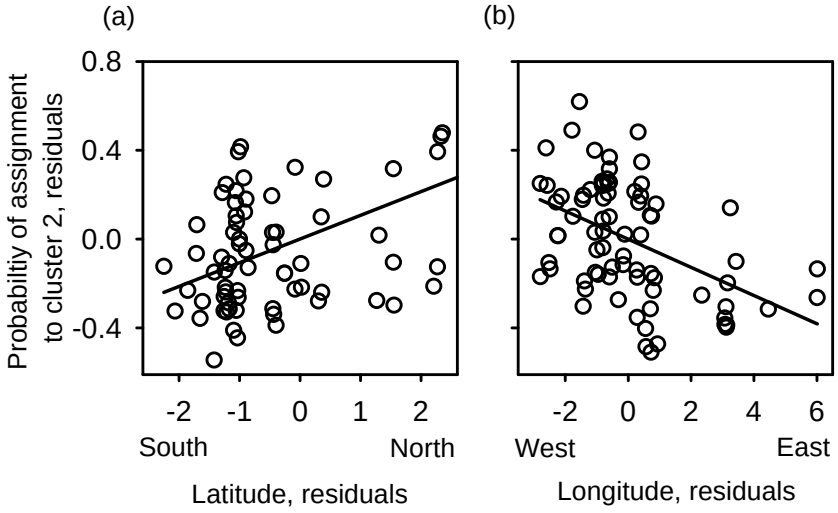

Figure 8. Partial regression plots of the relationship between the mean probability that an individual in a *C. defloratus* population belongs to cluster 2 and the significant explanatory variables (a) latitude and (b) longitude. For statistical analysis see text.

DISCUSSION

In agreement with the abundance centre model (Brown 1984) the size of populations of *C. defloratus* decreased and the isolation between them increased towards the northern range limit (Chapter 2). We also found the changes in population genetic diversity and differentiation across the studied area that is predicted as a result of this pattern of abundance (Ellstrand & Elam 1993, Lesica & Allendorf 1995). Genetic diversity within populations of *C. defloratus* decreased from the centre of the species distribution in the Alps towards the very disjunct populations at the northern range limit in Germany, whereas their distinctness increased. The differentiation between populations was higher at the range limit than at the centre. However, contemporary population size explained little of the patterns found.

Genetic structure among populations

In *C. defloratus* the molecular genetic differentiation between pairs of both central and peripheral populations increased strongly with their geographic distance, a pattern known as isolation by distance (IBD, Hutchison & Templeton 1999). Such a pattern indicates gene flow between neighbouring populations (Hutchison & Templeton 1999, Rousset 1997, Eckstein et al. 2006). However, the IBD pattern observed for peripheral populations cannot reflect current gene flow, because the peripheral populations are very disjunct and the dispersal of *C. defloratus* is poor. Because of its disjunct distribution, its cold tolerance and its virtual restriction to permanently treeless habitats *C. defloratus* is considered to be a glacial relict in

Central Europe outside of the Alps (Lange 1996). The genetic pattern observed may thus reflect the much higher gene flow during the last glaciation, when conditions were generally more favourable for the species at lower altitudes and the species was probably much more common outside of the Alps. With the spread of trees, the species would have become restricted to the current open, usually treeless habitat on rocks and cliffs, where the genetic structure was preserved due to the longevity of the species that reduces genetic drift. As *C. defloratus* is a clonal plant, it is potentially immortal. The Alps could have been recolonised from populations at the northern and southern edges of the Alps (see Schönswetter et al. 2002, 2005). Similar patterns of IBD in spite of strong current isolation between populations have been found for the long-lived ice age relict plants like *Saxifraga paniculata* (Reisch et al. 2003) and *Dodecatheon amethystinum* (Oberle & Schaal 2011).

However, our results also indicate that in spite of the longevity of the species, small and isolated populations were nevertheless affected by drift. The genetic variation of small populations was reduced and genetic differentiation between the more isolated peripheral populations increased more strongly with geographic distance than between central populations, indicating a stronger effect of genetic drift at the northern range limit of the species in line with the predictions of the ACM. This interpretation is also supported by the increase in mean genetic divergence towards the range margin, indicating that peripheral populations are more distinct. In contrast, in *Vaccinium stamineum* (Yakimowski & Eckert 2008) the genetic distinctness of populations was not related to their range position. Few studies have compared the slopes of the IBD relationships between peripheral and central populations. Hamilton & Eckert (2007) performed such an analysis and in agreement with our results they found significantly greater genetic distances between peripheral than between central populations of *Geum trifolium* at a given pairwise geographical distance. However, in contrast to our results the slopes of the regression lines did not differ significantly between population types. In a recent, study on the genetic structure of disjunct, edge and core populations of *Pulmonaria officinalis* (Meeus et al. 2012) significant IBD was found for disjunct and edge populations, but not for core populations. The authors suggest that historical processes influenced the genetic structure of *P. officinalis*.

Factors contributing to a significant pattern of IBD have been much discussed but are not well understood and generalisations are difficult (Moyle 2006, Kuss et al. 2008, Meirmans 2012). It has been assumed that the significance of the relationship of genetic distances between pairs of populations and their geographic distances and the value of the regression slope depends on the interplay of several factors like effective population size, persistence of populations,

physical barriers and dispersal ability of individuals, but also maximum geographic sampling distance (Garnier et al. 2004, Crispo & Hendry 2005, Moyle 2006, Kuss et al. 2008). The strong effect of IBD on the genetic data of *C. defloratus* sampled across the range of the species may explain why no clear clustering of the very disjunct northern or the other populations of *C. defloratus* was found. In a recent study it has been shown that the number of clusters can be overestimated if the data analysed by spatial Bayesian methods are characterised by IBD (Frantz et al. 2009). Nevertheless, in our study we detected only two clusters and the mean probability of assignment to a cluster was related to its range position.

Genetic diversity within populations

In line with the prediction of the ACM, the genetic diversity within populations of *C. defloratus* decreased towards the northern range limit. This continuous decline of genetic diversity is in contrast to the results of most other studies that analysed continuous changes in population genetic patterns across the distributional range of a species (ref. in Eckert et al. 2008, Yakimowski & Eckert 2008), and not only compared two population types (peripheral vs. central). However, a few other studies along gradients also found the patterns predicted by the ACM. Genetic diversity decreased with latitude towards the northern range limit of *Cirsium acaule* in Britain (Jump et al. 2003). At the southern hemisphere genetic diversity decreased from southern central populations of *Polylepis australis* in Argentina towards the northern peripheral populations suggesting an equatorward migration following past climatic changes (Hensen et al. 2011). In contrast to studies along gradients in range position, most of the studies comparing only a few central and peripheral populations found both lower genetic diversity and higher differentiation at the range margin (Eckert et al. 2008, but see Wagner et al. 2011).

The reasons for the different results based on continuous and categorical studies are not clear (Yakimowski & Eckert 2008). A possible explanation could be that in many species the abundance and thus genetic diversity does not decline continuously towards the range margin as predicted by the ACM, but that peripheral populations differ from all others (rare - periphery hypothesis, Gaston 2009).

The effect of population size on genetic diversity

The genetic diversity of populations of *C. defloratus* and their mean genetic distinctness were best explained by the range position (latitude) of a population. Genetic diversity was also related to the size of populations, but the correlation was weak although significant, and population size did not explain a significant amount of variation in addition to latitude. A possible explanation for the small influence of current population size on genetic diversity is that effective population size and the number of ramets may be only weakly related, because *C. defloratus* is a clonal species (see Wolf et al. 2000) and the number of genets is not known. Moreover, current population sizes may be different from those in the past, as has also been suggested for the long-lived *Gypsophila fastigiata* (Lönn & Prentice 2002) and the moss *Sphagnum angermanicum* (Urban et al. 2005). Similarly weak relationships between population size and genetic diversity across a species distribution as in *C. defloratus* have been also found in the long-lived *Gypsophila fastigiata* (Lönn & Prentice 2002). In most studies a positive relationship between genetic diversity in neutral genetic markers and plant population size has been found (Leimu et al. 2006).

CONCLUSIONS

We analysed changes in genetic diversity of populations and differentiation between them along a central-peripheral gradient in a long-lived species that is considered to be a glacial relict outside of the Alps. Analyses of AFLP markers indicated that in agreement with the abundant centre model the genetic structure of *C. defloratus* was influenced by the range position of populations, as genetic diversity of populations declined and differentiation between them increased with latitude, i.e. towards the range margin. However, our results suggest that patterns of genetic variation in long-lived species may be influenced more by patterns of historical gene flow than by current population size (Reisch et al. 2003, Oberle & Schaal 2011).

CHAPTER 5

Diversity and differentiation in quantitative traits of populations of an alpine plant varies along environmental gradients

in preparation
with Diethart Matthies

ABSTRACT

The abundant centre model (ACM) predicts that because of smaller size and greater isolation of populations at the range margin the genetic variability within populations will be lower and differentiation between populations higher than at the centre of the distribution of a species. These predictions have mostly been supported by studies of variation in neutral molecular markers, but have not been tested using evolutionary important quantitative traits. We sampled seeds from individuals of *Carduus defloratus* from 32 populations along a gradient from the centre of the species distribution in the European Alps to its northern range limit in the low mountains of central Germany. To study the variation in 18 quantitative traits and to compare it with that of AFLP markers plants from different seed families were grown up in a common garden. The genetic variability of quantitative traits was not related to neutral marker variation in populations, did not increase with population size, and for most traits did not decrease towards the range margin. Genetic differentiation between populations in many quantitative traits (Q_{ST}) was higher than that in molecular markers (Phi_{ST}), indicating divergent selection. Trait means differed among populations and were related to environmental gradients, indicating adaptive differentiation. Genetic distances between populations (Mahalanobis distances) based on vegetative and reproductive traits increased more strongly with geographical distance in peripheral than in other populations, indicating less differentiation in quantitative traits at the range periphery. Only genetic distances between populations based on vegetative, but not reproductive traits, were related to marker-based distances. The results indicate that patterns of genetic variation in quantitative traits which may be under selection do not follow the predictions of the ACM. Peripheral populations of plants may contain alleles important for the adaptation to changing conditions. The results also confirm that the variation in molecular markers is a poor predictor of variation in quantitative traits.

INTRODUCTION

Biogeographical models predict that populations become smaller, less dense, more fragmented, and less reproductive from the distribution centre of a species towards its range margin as conditions become less favourable (Brown 1984, Brown et al. 1996, Gaston 2009). The greater isolation and smaller size of peripheral populations should lead to less gene flow among populations and stronger genetic drift, resulting in higher differentiation among populations and lower within-population genetic diversity in peripheral regions (Hampe & Petit 2005, Eckert et al. 2008, Gaston 2009).

Populations at their range margins are often of special concern for conservation (Hamilton & Eckert 2007), but the conservation value of peripheral populations has been a matter of debate (Lesica & Allendorf 1992, 1995, Van Rossum et al. 2003). Because of stochastic loss of genetic variation, such populations may have low evolutionary potential (Lesica & Allendorf 1995, Vucetich & Waite 2003, Eckert et al. 2008) and because of their small size populations may be more strongly threatened by random environmental fluctuations (Matthies et al. 2004). It has therefore been argued that conservation efforts should be concentrated on large populations which are mostly situated in the centre of a distribution (Lesica &Allendorf 1995). However, selection regimes in peripheral populations are likely to be different from those in central populations and together with reduced gene flow this will lead to rapid genetic divergence and local adaptation in peripheral populations. It has thus been suggested that peripheral rather than central populations harbour the bulk of a species' genetic diversity (e.g. Petit et al. 2003, Hewitt 2004, Hampe & Petit 2005). Peripheral populations may harbour important adaptations (Lenormand 2002), may facilitate shifts in the geographical distribution of a species in response to climate change (Etterson & Shaw 2001, Kramer & Havens 2009), and may even be considered essential for the survival of a species (Safriel et al. 1994). In particular, disjunct populations, i.e. peripheral populations which are situated well outside the core of a species' distribution, may have a unique gene pool (Hamilton & Eckert 2007).

Comparisons of the genetic diversity of central and peripheral populations have almost exclusively been concerned with variation in molecular markers and there has been little effort to test whether the geographical trends in neutral variation are reflected by quantitative trait variation, which is likely to influence the adaptive potential of populations (Eckert et al. 2008, Kramer & Havens 2009, but see Volis et al. 1998). Large-scale quantitative genetic comparisons of peripheral vs. central populations could evaluate fundamental predictions of evolutionary theory as well as the conservation value of peripheral populations (Hamilton &

Eckert 2007), but are very rare (Sagarin & Gaines 2002, Angert 2006, Hamilton & Eckert 2007, Eckert et al. 2008).

Molecular and quantitative genetic variation are often only weakly correlated, because in contrast to variation in molecular markers which is supposed to be only influenced by drift, quantitative traits are usually also under selection (Reed & Frankham 2001, Volis et al. 2005, Leinonen et al. 2008). The role of selection as compared to drift for population differentiation can be assessed by a comparison between F_{ST}-values and their equivalent for quantitative traits (Q_{ST}-values; Steinger et al. 2002, Volis et al. 2005, Leinonen et al. 2008). There are three outcomes of such a study: (1) If $Q_{ST} > F_{ST}$, this is commonly interpreted as evidence for divergent selection and adaptation to local environments; (2) if Q_{ST} and F_{ST} are similar, genetic drift alone is thought to be sufficient for explaining the observed genetic differentiation; (3) if $Q_{ST} < F_{ST}$, this is interpreted as indicating convergent selection that favours the same genotypes at different sites (Volis et al. 2005). A recent review concluded that Q_{ST}-values are on average higher than F_{ST}-values, suggesting an important role for natural selection as a cause of population differentiation in quantitative traits (Leinonen et al. 2008). Quantitative might not exceed molecular genetic differentiation if selection has not had enough time to drive divergence (Whitlock & McCauley 1999). In the rare *Liatris scariosa* the small size of many of the studied populations may have resulted in reduced effectiveness of selection relative to random genetic drift (Gravuer et al. 2005). Evidence for unifying selection ($Q_{ST} < F_{ST}$) has been reported for the plants *Clarkia dudleyna* (mean Q_{ST}, Podolsky & Holtsford 1995), *Brassica insularis* (mean Q_{ST}, Petit et al. 2001), and for fitness related traits of *Scabiosa columbaria* from the Swiss Jura mountains (Scheepens et al. 2010).

Here we compare quantitative traits and their genetic variation obtained in a common garden experiment with plants of *Carduus defloratus* L. (Asteraceae) from 32 populations along a gradient from the centre to the periphery of the distribution of the species in Central Europe. As predicted by biogeographical models (Brown 1984, Gaston 2009) populations of *C. defloratus* in Central Europe become smaller, less dense, more fragmented, less reproductive (Chapter 2) and genetically less diverse at marker loci, but more strongly differentiated from their distribution centre in the Alps towards the northern range limit (Chapter 4). To evaluate the role of selection compared to drift we compare Q_{ST} and Phi_{ST} values (F_{ST} equivalent for dominant markers) and test for correlations between quantitative and molecular genetic distances between populations. Recently, Whitlock (2008) has criticised Q_{ST} vs. F_{ST} comparisons and advocated instead to relate population trait means of plants grown in a common garden to environmental gradients to detect patterns caused by selection. We thus

also relate trait means to environmental gradients as an alternative method to detect divergent selection.

We asked the following specific questions: (1) Does the genetic variation in quantitative genetic traits decrease towards the range margin and increase with population size? Is the quantitative genetic variation correlated with molecular genetic variation? (2) Is the quantitative genetic differentiation higher between peripheral than between central populations, and does it increase with geographical distance and molecular genetic differentiation between populations? (3) Is the genetic differentiation between populations higher for quantitative traits than for molecular markers?

MATERIALS AND METHODS

Study species

C. defloratus is a diploid, long-lived perennial with a mixed mating system. The clonal plant produces one or more rosettes. From June to October the rosettes produce one ore more inflorescences, each with a single flowerhead (capitulum). The achenes posses a pappus and are dispersed by wind. However, dispersal by wind is probably very limited because the achenes are large (2 mg). Dispersal in the related species *C. nutans* and *C. acanthoides* is usually less than 5 m (Skarpaas & Shea 2007).

In Central Europe *C. defloratus* occurs from the Alps in the south to the central German low mountain ranges in the north. It also occurs south of the Alps in the Italian Appenines, further southwest in the Pyrenees and further east in the Tatra mountains (Meusel & Jäger 1992). In the Alps *C. defloratus* is a common species of alpine grassland and on rocks at higher altitudes, where it covers large, continuous areas. In contrast, towards the northern range limit the species becomes increasingly rare and is restricted to rocks, cliffs and open woodlands where trees are scarce, but partially shading the populations. The altitudinal range of *C. defloratus* is large, from 300 m at the northern range limit to 2600 m in the Alps (Landolt 2003).

Field measurements and seed sampling

In summer 2006 we selected 32 populations of *C. defloratus* in Central Europe along a gradient from the distribution centre in the Alps to the northern peripheral populations in the low mountains of Hesse and Thuringia (Fig. 1). This gradient is both a gradient of increasing

latitude (46.5 - 51.3 °N) and decreasing altitude (373 - 1611). Because altitude and latitude were highly correlated ($r = -0.81$), we used principle component analysis to reduce the two geographical variables to a single principle component ("centrality") which increased with altitude and decreased with latitude. With centrality, mean temperature decreased ($r = -0.76$, $p < 0.001$) and precipitation ($r = 0.95$, $p < 0.001$) increased strongly. The temperature and precipitation data were obtained from the WorldClim database (Hijmans et al 2005). We related the differences in mean annual temperature and precipitation between pairs of populations within population types to their geographical distance and evaluated the correlations by Mantel tests (Mantel 1967). The correlation between temperature distance and geographic distance between pairs of populations of *C. defloratus* was significant ($r = 0.40$, $p < 0.001$) as was the correlation between precipitation distance and geographic distance ($r = 0.48$, $p < 0.001$).

For certain analyses, the populations were divided into three groups: (1) Peripheral populations from the northern range limit in the Werrabergland, Ohmgebirge and Ilm-Saale-Platte regions, (2) central populations from the Alps, and (3) intermediate populations from the regions in between, i.e. the Franconian Alb, Swabian Alb and Swiss Jura (Fig. 1).

For each population, its longitude, latitude, and altitude were determined with a GPS. We determined population size as the number of flowering ramets. In each population three to five plots of 1 x 1 m were selected at random at their densest part. Within each plot we estimated the total cover of all vascular plants, the height of flowering ramets of *C. defloratus* and measured the length of the longest leaf. For the later determination of the specific leaf area (SLA) one fully developed leaf was sampled from six to 22 individuals per population, pressed and dried between sheets of paper. Ripe fruitheads were sampled from 10-15 plants, if available. We only sampled leaves from plants that were not chosen for fruithead sampling.
At the end of May 2007 seeds were germinated and 2 - 10 (median, 3) randomly selected seedlings per family were transplanted individually into 2 l plastic pots filled with a mixture of 80% nutrient-poor commercial soil (TKS 1, Floragard Vertriebs GmbH, Oldenburg) and 20% sand, and placed into flower beds in the Botanical Garden of the University of Marburg. In total, we transplanted 905 seedlings of *C. defloratus* from 307 seed families and 32 populations.

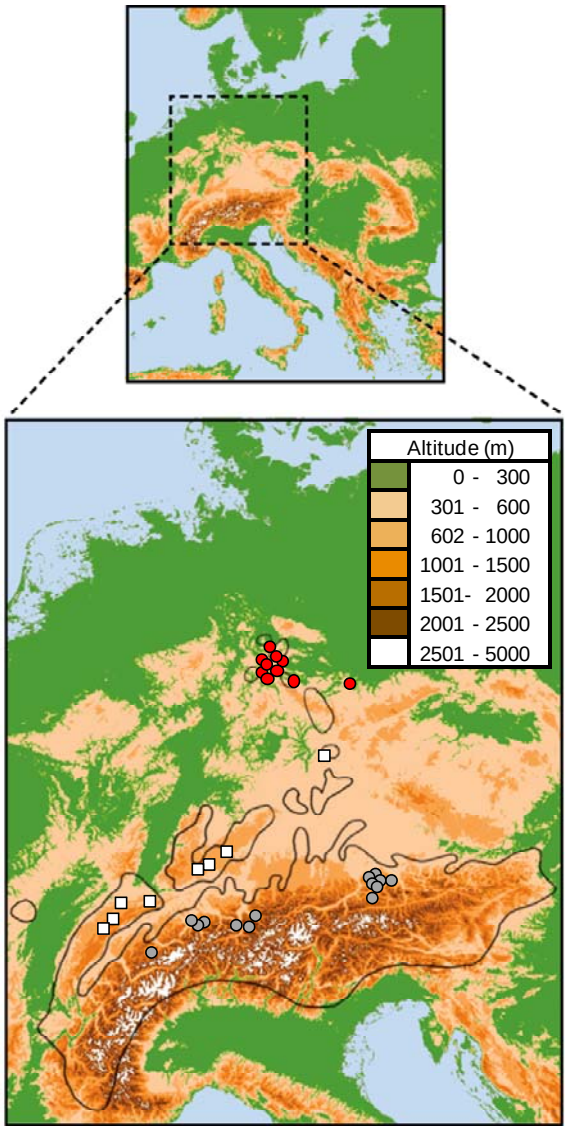

Figure 1. Map of the distribution of the alpine thistle *Carduus defloratus* ssp. *defloratus* in Central Europe (after Meusel & Jäger 1992). The distribution area is bounded by the irregular black line. The circles indicate the location of the 32 studied populations. Red dots indicate the 10 peripheral populations from the northern range limit in the Werrabergland, Ohmgebirge and Ilm-Saale-Platte regions; gray dots indicate the locations of the 14 central populations from the Alps; white squares indicate the locations of the eight intermediate populations from the regions in between i.e. the Franconian Alb, Swabian Alb and Swiss Jura.

In August 2007 (after 12 weeks of growth) we recorded the length and width of the largest leaf for every plant as a measurement of plant size. Leaf chlorophyll content of this leaf was measured using a hand-held chlorophyll meter (SPAD-502, Minolta) and converted into chlorophyll concentration using a calibration equation by Richardson et al. (2002). We sampled one fully developed rosette leaf of each individual. The leaves were dried between sheets of paper, scanned, and weighed individually. From the scanned images the total area

and the perimeter of the leaves were determined with the ImageJ 1.40 software (http://rsb.info.nih.gov/ij/). Moreover, the length of one spine was measured at the widest part of the left half of the leaf. To determine the depth of the incision of leaves, we measured the distance from the tip of the longest lobe to its base.

In summer 2008 the date of first flowering of each individual was recorded as number of days since 1 January 2008. Several variables related to the size of flowerheads (diameter of capitulum basis, capitulum height, petal length) were measured for each plant as soon as it flowered. In July 2008 the number of rosettes, rosette leaves and flowerheads were counted for each plant, and the following traits measured: the length of each inflorescence stalk, the number of leaves of the longest shoot, and the distance between the uppermost leaf and the flowerhead (leaf capitulum distance). Three further variables were derived from these data: inflorescence height as the length of the longest inflorescence stalk, cumulative stalk length as the sum of all inflorescence stalks, and the mean length of an internodium as inflorescence height divided by the number of leaves along the shoot.

Statistical analysis

To obtain factors that combine several correlated vegetative and reproductive traits, we used principle component analysis. The first vegetative principle component (LEAFSIZE) explained 21.2% of the variation and was strongly related to traits that determine leaf size, like leaf length and width (Table 1). The second component (PLANTSIZE, 20.2%) was strongly related to traits measuring vegetative size like the number of rosettes and leaves. The first reproductive principle component (CAPITSIZE) explained 22% of the variation and was related to traits describing the size of the capitula. The reproductive principle component (INFLSIZE) explained 21.5% of the variation and was related to traits that measure the size of the inflorescence like the number of capitula and stalk length, but it was also negatively related to time until flowering.

Table 1. Loadings of vegetative and reproductive traits of *Carduus defloratus* on principal components derived from (a) vegetative and (b) reproductive traits. The strongest correlations (r > 0.45) are in bold-face.

	PC1 (LEAFSIZE)	PC2 (PLANTSIZE)
(a) *Vegetative traits*		
Leaf length	**0.87**	0.11
Leaf width	**0.86**	-0.08
Leaf perimeter	**0.56**	-0.08
Number of rosettes	-0.04	**0.91**
Number of leaves	0.04	**0.92**
Specific leaf area	-0.24	0.16
Spine length	0.21	-0.08
Incisiveness	-0.02	0.15
Chlorophyll content	-0.07	0.22
(b) *Reproductive traits*	PC1 (CAPITSIZE)	PC2 (INFLSIZE)
Diameter of capitulum basis	**0.71**	-0.27
Capitulum height	**0.75**	0.02
Petal length	**0.61**	-0.05
Date of first flowering	**-0.56**	-0.46
Number of capitula	-0.22	**0.91**
Cumulative stalk length	0.05	**0.87**
Inflorescence height	0.37	0.24
Length of an internodium	-0.12	0.08
Distance uppermost leaf - capitulum	0.11	0.00

To analyse variation among populations of *C. defloratus* in quantitative traits we used two approaches. We compared trait means for the three population types (peripheral, intermediate, central) by analyses of variance of population means and we analysed which variables influenced trait means through multiple regression analyses. Potential explanatory variables were centrality (i.e. position within the central-peripheral gradient), longitude, vegetation cover, and population size. The strength of evidence for each possible model was assessed by the Bayesian information criterion (BIC). In the results we present the models with the lowest BIC, but only those that were significant. Population size was log-transformed prior to analysis.

We tested for significant variability of traits among populations and families within populations using nested analyses of variance. Variance components for populations (V_{Pop}), families within populations (V_{Fam}) and individuals within families (V_{Res}) were calculated by

restricted maximum likelihood (REML) using the varcomp function (package ape) and lme function (package nlme) of R 2.12.1 (R Development Core Team 2011).

The calculated variance components were the basis for the following estimation of quantitative genetic measures. To assess the genetic variation of populations we estimated the heritability (h^2) and the genetic coefficient of variation ($CV_{genetic}$, also called evolvability, Houle 1992) for each trait. The genetic differentiation among populations in quantitative traits was assessed by Q_{ST}. Since the paternity of the seeds collected from a flowerhead is not known and *C. defloratus* is self-compatible we assumed that members of a seed family are on average related as full-sibs rather than half-sibs, which is considered conservative (Podolsky & Holtsford 1995, Storfer 1996). We also assumed that maternal effects are weak and that most of the genetic variance was additive (cf. Waldmann &Andersson 1998). We thus used the following formulas (Houle 1992, Waldmann & Andersson 1998, Jimenez-Ambriz et al. 2007):

(i) $h^2 = 2V_{Fam} / (V_{Fam} + V_{Res})$

(ii) $CV_{genetic} = SQRT(2*V_{Fam}) / Mean$

(iii) $Q_{ST} = V_{Pop} / [(4\ V_{Fam} + V_{Pop})]$

$CV_{genetic}$ and h^2 were strongly correlated for all traits ($r = 0.72 – 0.97$, $p < 0.0001$). As it has been argued that $CV_{genetic}$ is a more suitable measure of evolutionary potential (Houle 1992, Hansen et al. 2011), we present in the following only the results for $CV_{genetic}$. We estimated the 95% confidence interval for each Q_{ST} by the jackknife procedure over populations following O'Hara and Merilä (2005). We analysed the relationship between evolvability and possible explanatory variables (vegetation cover, centrality, longitude, population size) by multiple regressions and present the results of the models with the lowest BIC, but only those that were significant. We also analysed the relationship between evolvability and molecular genetic variation in populations by correlating the $CV_{genetic}$-values for traits with Nei's gene diversity corrected for sample size based on AFLP markers (see Chapter 4). For the calculation of Nei's gene diversity, we identified and excluded loci that are putatively under selection using BAYESCAN 2.01 (Foll et al. 2008) with the false discovery rate set to 0.001.

We compared the genetic differentiation between populations for quantitative traits with the overall molecular genetic differentiation between populations by comparing the Q_{ST}-values for the various quantitative traits with the overall Phi_{ST}-value derived from an analysis of molecular variance (AMOVA, Excoffier et al. 1992).

To estimate the quantitative genetic differentiation between populations we calculated Mahalanobis distances (Knapp &Rice 1998, Bekessy et al. 2003, Gravuer et al. 2005) between populations based on family means of vegetative and reproductive traits. Mahalanobis distances measure distances in multivariate space taking into account correlations among traits and are independent of the scale of the traits (Legendre &Legendre 1998). To analyse the relationship between quantitative genetic distances between populations and pairwise geographic distances, and to compare the relationships among the three types of populations, we used general linear models relating the pairwise Mahalanobis distances between populations within the three groups to log-geographical distance, the factor population type, and their interaction. We further partitioned the factor population type into the two contrasts peripheral vs. other populations, and central vs. intermediate populations. The significance of terms was determined with permutation tests (10000 permutations), using an R-script. We derived the reported p-values from the proportion of times the F-values for permutated datasets exceeded the observed values. In an analogous way we tested for differences in relationships between quantitative and molecular genetic distances that had been estimated as linearised Phi_{ST}-values (Rousset, 1997, Peakall & Smouse 2005, see Chapter 4).

RESULTS

Comparison of quantitative traits among population types

In the common garden, all vegetative and reproductive traits and the first two principle components extracted from each group of traits (Fig. 2a,b) varied significantly among populations (all $p < 0.05$), and with the exception of internodium length, all traits also varied among families within populations (all $p < 0.05$). Plants from the three types of populations also differed in a number of traits in the common garden. Plants from the peripheral northern populations produced larger leaves than plants from the other population types (Table 2a,b). In contrast, plants from central (alpine) populations produced more rosettes and leaves, but smaller capitula on lower flower stalks. Moreover the form of leaves of plants from central populations differed from that of other plants, as they were more strongly incised.

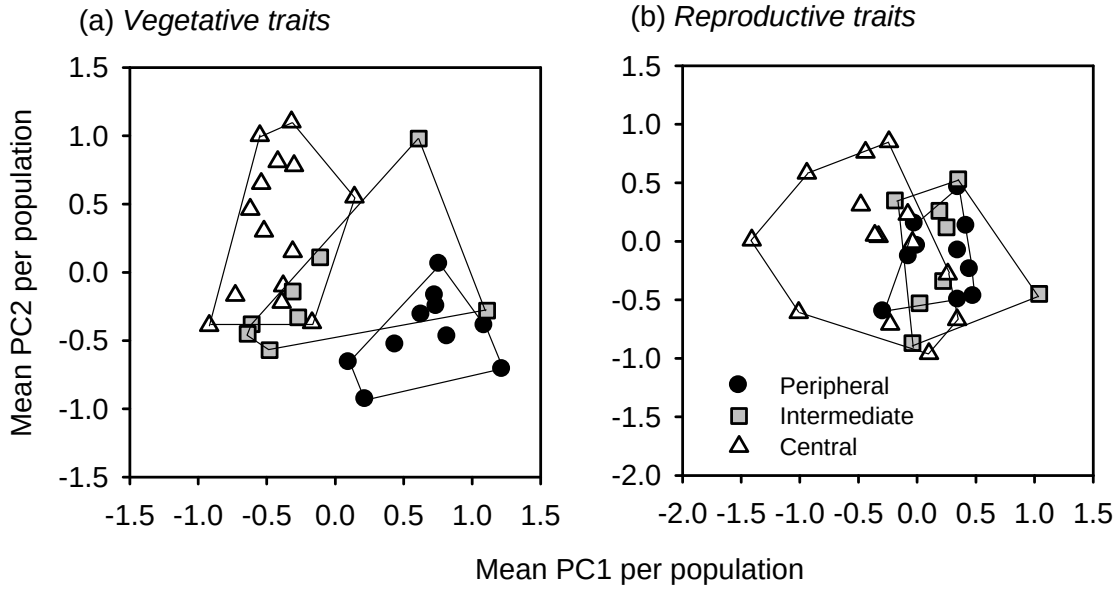

Figure 2. Illustration of the mean principle components averaged among individuals, families and among families within each of 32 populations of *Carduus defloratus* for (a) vegetative and (b) reproductive traits. The northern peripheral, the intermediate, and the southern central populations are delimited by minimum convex polygons.

Table 2: Trait means for plants from peripheral, intermediate, and central populations of *Carduus defloratus* grown in a common garden.

	Population type			
	Peri.	Inter.	Central	$F_{2,29}$
(a) *Vegetative traits*				
Leaf length (cm)	15.2^{A}	13.2^{B}	12.9^{B}	15.83***
Leaf width (cm)	5.9^{A}	5.2^{B}	4.9^{B}	13.83***
Number of rosettes	1.5^{A}	$1.9^{A,B}$	2.3^{B}	7.89**
Number of leaves	19.3^{A}	21.3^{A}	26.2^{B}	8.49**
Specific leaf area ($cm^2\ mg^{-1}$)	11.2^{A}	$11.3^{A,B}$	12.2^{B}	4.98*
Spine length (mm)	2.1	1.9	2.0	1.23
Incisiveness (mm)	5.0^{A}	4.6^{A}	6.8^{B}	8.65**
Chlorophyll content ($\mu g\ cm^{-2}$)	40.5	44.5	42.3	1.26
Leaf perimeter (cm)	67.9^{A}	56.8^{B}	57.1^{B}	9.68***
$PC1_{veg}$ (LEAFSIZE)	5.7^{A}	4.9^{B}	4.6^{B}	22.53***
$PC2_{veg}$ (PLANTSIZE)	4.6^{A}	$4.9^{A,B}$	5.3^{B}	8.64**
(b) *Reproductive traits*				
Inflorescence height (cm)	50.1^{A}	48.1^{A}	41.7^{B}	8.65**
Internodium length (cm)	5.4	5.7	5.6	0.59
Cumulative stalk length (cm)	83.7	84.5	80.7	0.15
Leaf - capitulum distance (cm)	12.3	13.3	12.8	0.52
Number of capitula	2.4	2.5	2.6	0.81
Diameter of capitulum basis (mm)	$11.1^{A,B}$	11.4^{A}	10.4^{B}	4.04*
Capitulum height (mm)	12.1^{A}	$12.0^{A,B}$	11.5^{B}	5.44**
Petal length (mm)	9.4^{A}	9.6^{A}	8.9^{B}	8.21**
Time until flowering (Julian day)	155	154	154	0.08
$PC1_{repro}$ (CAPITSIZE)	5.2^{A}	5.2^{A}	4.7^{B}	7.48**
$PC2_{repro}$ (INFLSIZE)	4.9	4.9	5.0	0.16

Clinal variation in quantitative traits

In multiple regression analyses, most of the vegetative traits and the two principle components were related to the centrality of a population, and some to its longitude (Table 3a), while the size of the population of origin had no influence. Leaf length, leaf width, leaf perimeter and the principle component LEAFSIZE (Fig. 3a) decreased with centrality indicating decreasing leaf size towards the range centre of *C. defloratus*. In contrast, the number of rosettes and leaves and $PC2_{veg}$ (Fig. 3b,c) increased both with longitude east and

centrality indicating increasing vegetative plant size from the periphery towards the centre of the distribution and from west to east. Incisiveness of leaves also increased with centrality and with longitude (Fig. 3 d,e). Specific leaf area, spine length, and chlorophyll content were not significantly related to any of the explanatory variables.

Table 3. The effects of the range position of a population (centrality factor) and other characteristics on (a) mean vegetative and (b) mean reproductive traits and their principle components in populations of *Carduus defloratus.* We present the models for which the Bayesian information criterion is minimal. Potential explanatory variables were population size, centrality, and longitude. For the other vegetative traits (specific leaf area, spine length, chlorophyll content) and reproductive traits (internodium length, cumulative stalk length, leaf-capitulum distance, and time until flowering) no significant relationships with any of the explanatory variables were found.

(a) *Vegetative traits*

Dependent variable	df	r^2	F	Explanatory variable	β	t
Leaf length	30	0.53	33.77***	Centrality	-0.73	-5.81***
Leaf width	30	0.60	45.84***	Centrality	-0.78	-6.77***
Number of rosettes	29	0.50	14.33***	Longitude	0.63	4.60***
				Centrality	0.51	3.76***
Number of leaves	29	0.49	13.96***	Longitude	0.61	4.45***
				Centrality	0.52	3.34***
Incisiveness	29	0.29	5.82**	Centrality	0.51	3.13**
				Longitude	0.34	2.07*
Perimeter	30	0.33	14.64***	Centrality	-0.57	3.83***
$PC1_{veg}$ (LEAFSIZE)	30	0.71	75.93***	Centrality	-0.85	-8.71***
$PC2_{veg}$ (PLANTSIZE)	29	0.51	15.16***	Longitude	0.62	4.62***
				Centrality	0.54	4.01***

Notes: * $p < 0.05$; **, $p < 0.01$; ***$p < 0.001$.

Table 3 continued

(b) *Reproductive traits*

Dependent variable	df	r^2	F	Explanatory variable	β	t
Inflorescence height	28	0.61	14.74***	Centrality	-0.68	-5.62***
				Longitude	-0.37	-2.93**
				Log population size	-0.26	-2.10*
Number of capitula	30	0.32	14.25***	Longitude	0.57	3.78***
Diameter of capitulum basis	29	0.39	9.08***	Longitude	-0.62	-4.11***
				Centrality	-0.36	-2.50*
Capitulum height	29	0.22	8.39**	Centrality	-0.47	-2.90**
Petal length	30	0.13	4.31*	Centrality	-0.36	-2.09*
$PC1_{repro}$ (CAPITSIZE)	29	0.27	5.42**	Centrality	-0.48	-2.91**
				Longitude	-0.36	-2.19*
$PC2_{repro}$ (INFLSIZE)	30	0.30	12.85**	Longitude	0.55	3.58**

Notes: * $p < 0.05$; **, $p < 0.01$; ***$p < 0.001$.

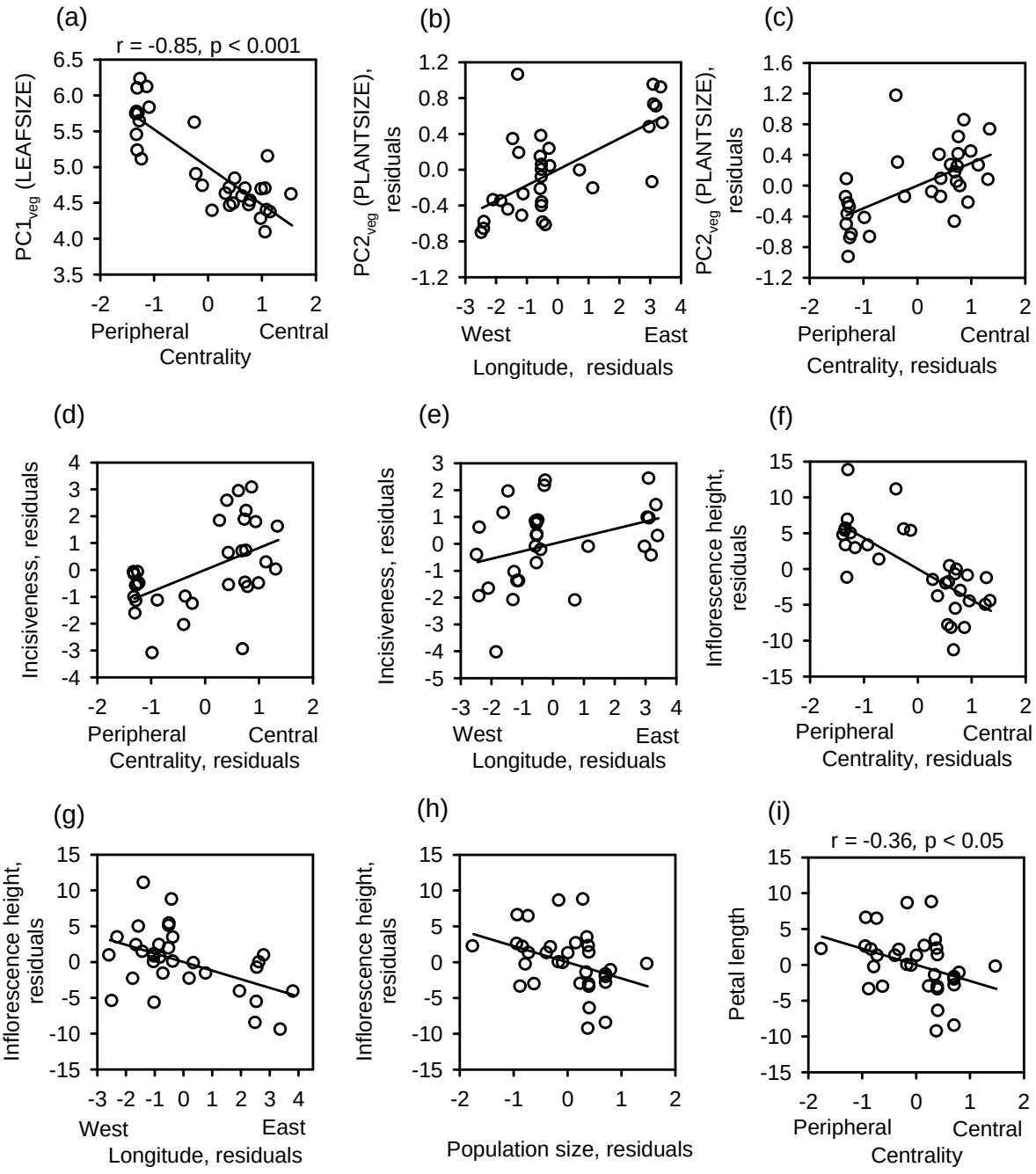

Figure 3. Relationships between (a,b) leaf incisiveness, (c) $PC1_{veg}$ (LEAFSIZE), (d,e) $PC2_{veg}$ (PLANTSIZE), (f,g,h) inflorescence height, and (i) petal length of *Carduus defloratus* and significant explanatory variables. Shown are regression plots (c, i) in the case of single explanatory variables and partial regression plots (a, b, d-h) in the case of several significant explanatory variables. For statistical analysis see Table 3.

Fewer reproductive than vegetative traits were related to centrality (Table 3b, Fig. 3f-i). Inflorescence height, capitulum height, petal length and the PC CAPITSIZE decreased significantly with centrality (Table 3b, Fig. 3 f-h). Capitulum size, the $PC1_{repro}$ (CAPITSIZE) and inflorescence height were lower in plants from eastern than from western populations, while the number of capitula and $PC2_{repro}$ (INFLSIZE) increased with longitude east. The size

of a population only explained a significant additional part of the variation for inflorescence height. Internodium length, cumulative stalk length, leaf-capitulum distance, and time until flowering were not significantly related to any of the explanatory variables.

Relationship between traits measured in field populations and in the common garden

Five traits were measured both for the field populations and their offspring in the common garden. Mean inflorescence height in the common garden and the field were positively correlated for the populations of *C. defloratus* (Fig. 4a), and there was a quadratic relationship between the mean length of leaves in the two environments (Fig. 4b). In contrast, no significant relationship between measures in the field and the garden were found for leaf perimeter ($r = 0.16$, $p = 0.39$), specific leaf area ($r = -0.01$, $p = 0.96$) and number of capitula per plant ($r = 0.04$, $p = 0.81$).

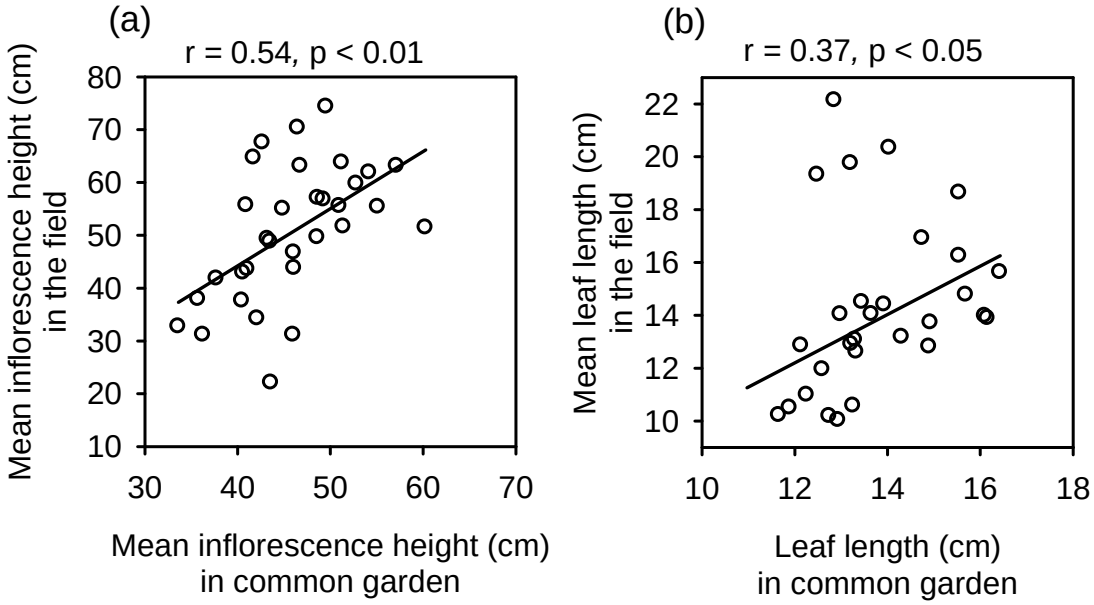

Figure 4. Relationship between population means of (a) inflorescence height of *Carduus defloratus* and (b) leaf length measured in the field and in the common garden.

Patterns in quantitative genetic variation

Various explanatory variables influenced the variation in quantitative genetic traits within populations, measured as evolvability ($CV_{genetic}$) of vegetative and reproductive traits (Table 4ab).

The evolvability of leaf width, the $PC1_{veg}$ LEAFSIZE, and of variables related to inflorescence size (inflorescence height, cumulative stalk length, $PC2_{repro}$ INFLSIZE) increased with the centrality of a population (Table 4a, Fig. 5a,b), while the evolvability of internodium length decreased with longitude east. The variation in the number of leaves was higher in small than in large populations. The $CV_{genetic}$ of the other vegetative and reproductive traits was not related significantly to any of the explanatory variables.

Table 4. The effects of the range of the species (centrality factor) and population characteristics on evolvability, i.e. genetic coefficients of variation ($CV_{genetic}$) of (a) vegetative and (b) reproductive traits and their principle components in populations of *Carduus defloratus.* We present the models for which the Bayesian information criterion is minimal. Potential explanatory variables were population size, centrality, and longitude. For the $CV_{genetic}$ of other vegetative traits (leaf width, leaf number, specific leaf area, spine length, incisiveness, chlorophyll content, leaf perimeter, $PC2_{veg}$) and reproductive traits (leaf-capitulum distance, number of capitula, diameter of capitulum basis, capitulum height, petal length, time until flowering, $PC1_{repro}$) no significant relationships with any of the explanatory variables were found.

Dependent variable	df	r^2	F	Explanatory variable	β	t
(a) *$CV_{genetic}$ of vegetative traits*						
Leaf width	30	0.17	6.30**	Centrality	0.42	2.51*
Number of leaves	30	0.21	7.74**	Log population size	-0.45	-2.78**
$PC1_{veg}$ (LEAFSIZE)	30	0.19	6.79*	Centrality	0.43	2.6*
(b) *$CV_{genetic}$ of reproductive traits*						
Inflorescence height	30	0.16	5.51*	Centrality	0.39	2.35*
Internodium length	30	0.21	8.05**	Longitude	-0.46	-2.84**
Cumulative stalk length	30	0.23	8.98**	Centrality	0.48	3.00**
$PC2_{repro}$ (INFLSIZE)	30	0.17	5.99*	Centrality	0.41	2.45*

Notes: (*), $p < 0.10$; * $p < 0.05$; **, $p < 0.01$.

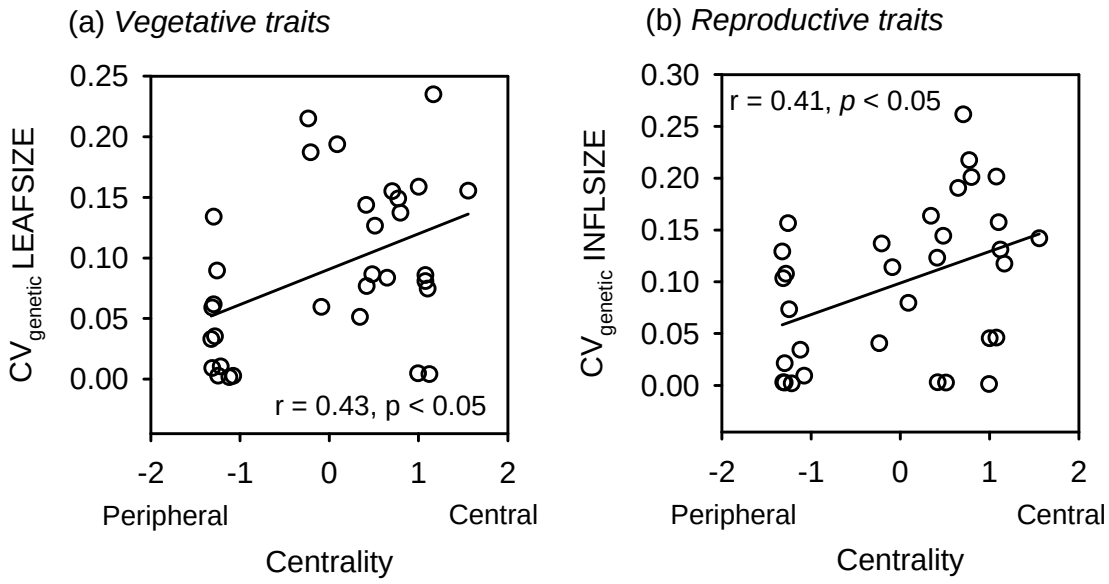

Figure 5. The relationships between (a) quantitative genetic variation in $PC1_{veg}$ (LEAFSIZE), and (b) quantitative genetic variation in $PC2_{repro}$ (INFLSIZE) and the range position of populations (centrality) within the Central European distribution area of *Carduus defloratus*.

Among the vegetative traits the highest evolvability was found for the incisiveness of leaves, and the number of rosettes and leaves; the lowest for leaf length, leaf perimeter and the two principle components PLANTSIZE and LEAFSIZE (Fig. 6a).. Among the reproductive traits evolvability was highest for cumulative stalk length, the distance between the uppermost leaf and the capitulum, and the number of capitula, whereas it was lowest for time until flowering and petal length (Fig. 6b).

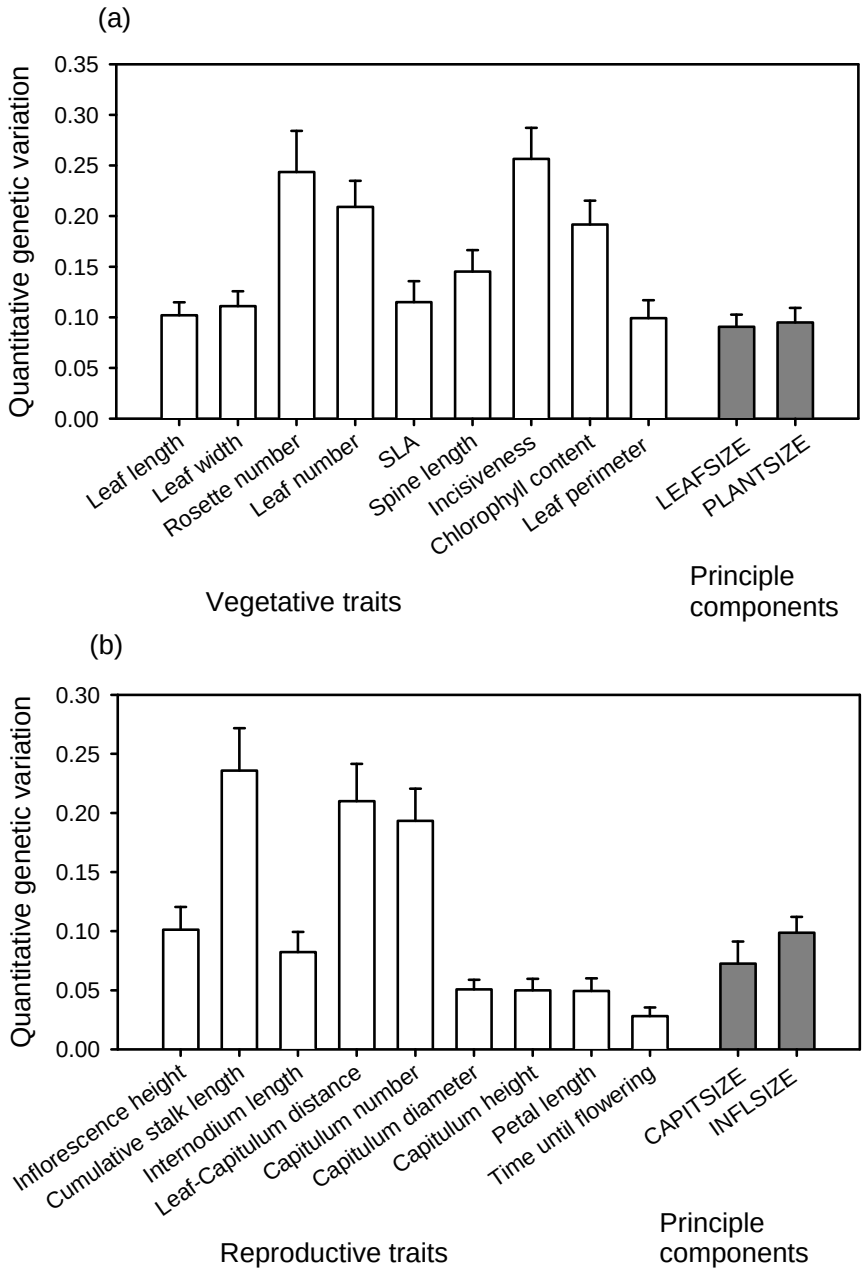

Figure 6. Mean evolvability ($CV_{genetic}$) and mean principle components of (a) vegetative and (b) reproductive traits of *Carduus defloratus.* Means over 32 populations +1 standard error.

Association between quantitative and molecular genetic variation

We tested for an association between the variation in quantitative genetic traits within populations, measured as evolvability ($CV_{genetic}$), and the molecular genetic variation, measured as Nei's gene diversity. Overall, there was no significant correlation between genetic variation in quantitative and molecular traits. The correlation between the mean evolvability of vegetative traits and gene diversity for all populations was weak ($r = 0.10$, $p = 0.59$; Fig. 7a) and correlations calculated separately for central, intermediate and peripheral

populations were also not significant (all $p > 0.22$). The correlation between the variation in reproductive traits and gene diversity was higher, but also not significant ($r = 0.29$, $p = 0.10$; Fig. 7b). Of the four principal components, only LEAFSIZE was related to gene diversity ($r = 0.48$, $p < 0.01$).

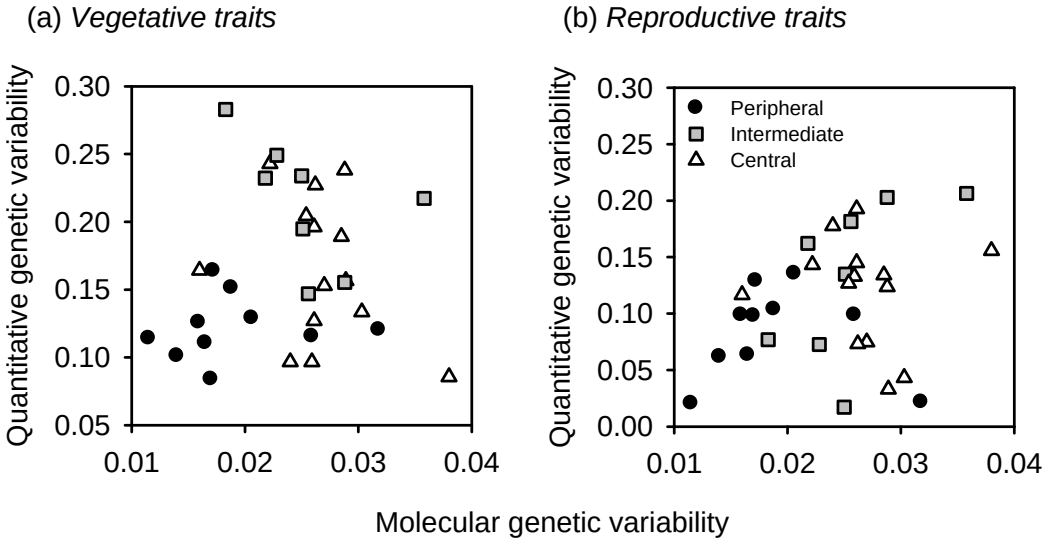

Figure 7. The relationship between mean quantitative genetic variability (mean $CV_{genetic}$ over traits) and molecular genetic variability for peripheral, intermediate and central populations of *Carduus defloratus.* (a) Vegetative traits ($r = 0.10$, $p = 0.59$), (b) reproductive traits ($r = 0.29$, $p = 0.10$).

Comparison of quantitative and molecular genetic differentiation

Q_{ST} -values of most vegetative traits, their principle components and of capitulum diameter were higher than the global Phi_{ST}-value, indicating that these traits are under divergent selection (Fig. 8). Q_{ST}-values of several vegetative traits and all the other reproductive traits had large confidence intervals including the Phi_{ST}-value. Some Q_{ST}-values like those for chlorophyll content, capitulum height, petal length, and time until flowering were very similar to the Phi_{ST}-value, suggesting that genetic drift might be sufficient to explain the differentiation in these traits.

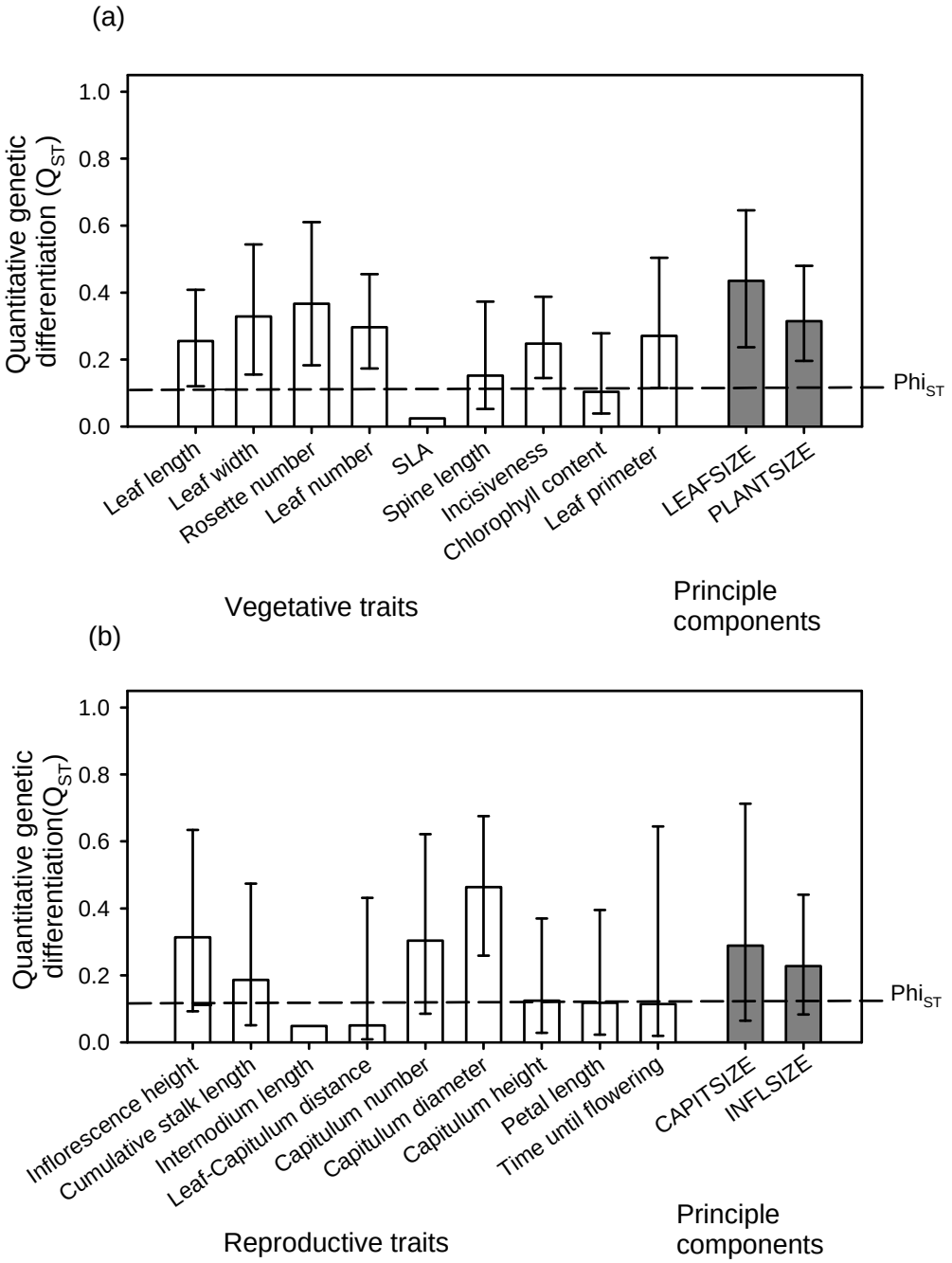

Figure 8. Quantitative genetic differentiation between populations (Q_{ST}) of *Carduus defloratus* for (a) vegetative and (b) reproductive traits and their principle components. For comparison between quantitative and molecular genetic differentiation the global Phi_{ST} value is shown (dashed line). Error bars indicate the confidence interval. Confidence intervals could not be calculated for the two lowest Q_{ST}-values.

Relationship between quantitative genetic differentiation, geographical distance and molecular genetic differentiation

The quantitative genetic differentiation between pairs of populations of *C. defloratus* was estimated by their Mahalanobis distance based on vegetative and reproductive traits. The genetic differentiation between pairs of populations based on both vegetative and reproductive traits increased with their geographical distance (Table 5a, Figure 9a,b). The genetic differentiation between populations increased far more strongly with geographic distance for intermediate and central than for peripheral populations. At distances > 5 km for vegetative traits and > 10 km for reproductive traits genetic differentiation was on average higher for intermediate and central than for peripheral populations.

Table 5. Results of general linear models of the effect of (a) pairwise geographical distance or (b) molecular genetic distance, population type (peripheral, intermediate or central) on quantitative genetic distances (Mahalanobis distances) between pairs of populations of *Carduus defloratus* based on vegetative or reproductive traits. P-values were obtained by permutation tests using 10 000 random permutations.

(a)

		Vegetative traits		Reproductive traits	
Source of variation	df	F	p	F	p
Geographic distance	1	65.5	< 0.001	101.1	< 0.001
Population type	2	10.9	< 0.001	6.5	< 0.010
Peripheral vs. others	1	7.1	< 0.01	0.6	0.44
Central vs. intermediate	1	14.6	< 0.001	12.4	< 0.001
Geographic distance x Type	2	8.7	< 0.001	9.3	< 0.001
Distance x peripheral vs. others	1	10.1	< 0.01	17.2	< 0.001
Distance x central vs. intermediate	1	7.4	< 0.01	1.5	0.23

Table 5 continued

(b)

		Vegetative traits		Reproductive traits	
Source of variation	df	F	p	F	p
Genetic distance	1	52.0	< 0.001	< 0.1	0.770
Population type	2	17.9	< 0.001	18.7	< 0.001
Peripheral vs. others	1	34.5	< 0.001	28.3	< 0.001
Central vs. intermediate	1	1.3	0.18	9.1	< 0.01
Genetic distance x Type	2	3.6	< 0.05	0.9	0.41
Gen. distance x peripheral vs. others	1	1.8	0.13	< 0.1	0.92
Gen. distance x central vs. intermediate	1	5.4	< 0.05	1.8	0.18

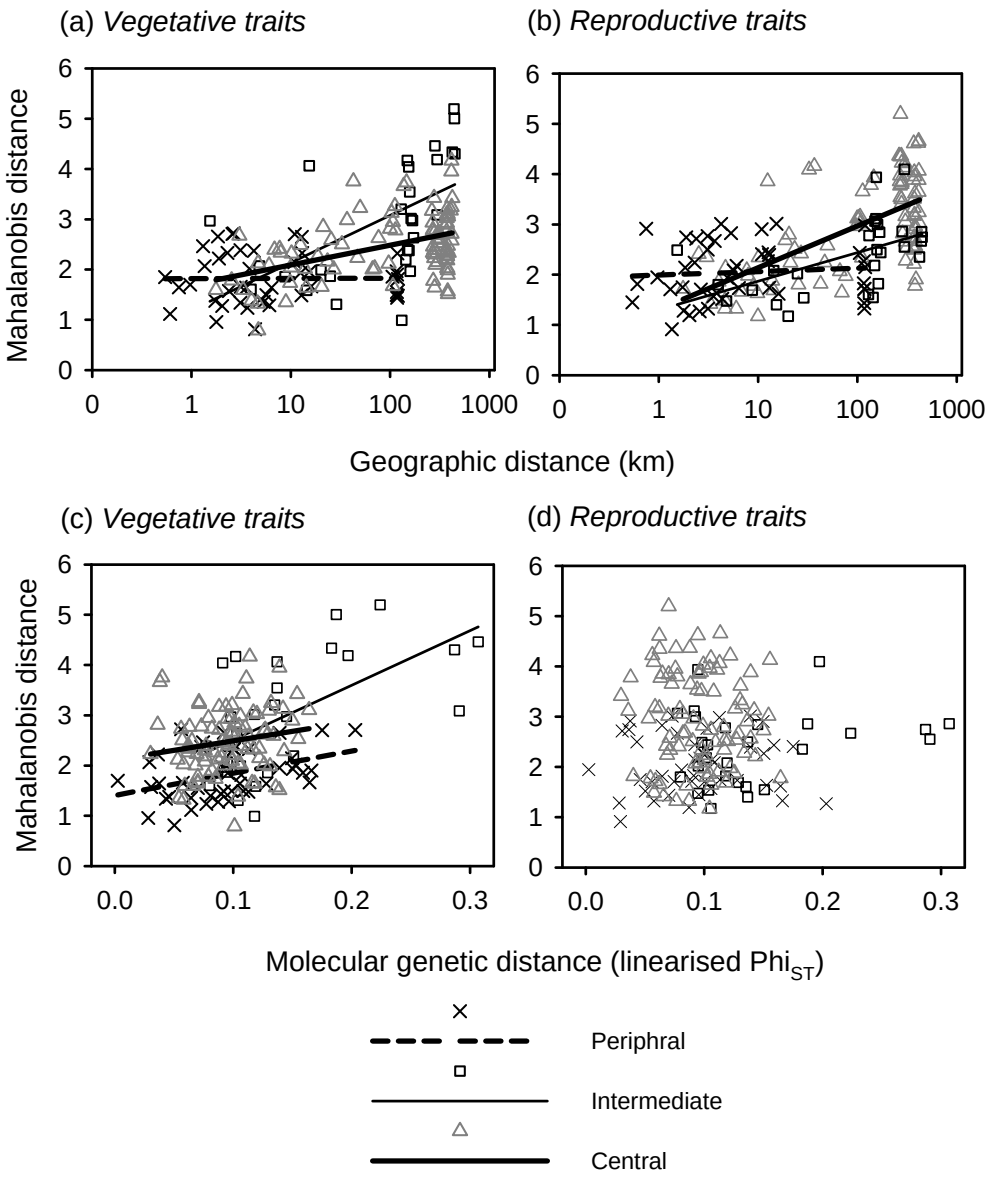

Figure 9. (a,b) The relationship between Mahalanobis distances between populations of *Carduus defloratus* of different types based on vegetative (a) and reproductive (b) traits and the geographic distance between populations, and the relationship between Mahalanobis distances based on vegetative (c) and reproductive (d) traits and pairwise linearised Phi_{ST} values.

The quantitative genetic distance between populations based on vegetative traits was positively correlated with their molecular genetic distance (Table 5b, Fig. 9c), but the increase was less strong for peripheral than for the other populations. There was no significant relation between the quantitative genetic distance between populations based on reproductive traits and their molecular genetic distance (Fig. 9d). In contrast, Mahalanobis distances between populations within population types were related to differences in mean annual temperature (vegetative traits: $r = 0.31$, $p < 0.001$; reproductive traits: $r = 0.23$, $p < 0.001$; Mantel test) and

precipitation (vegetative traits: $r = 0.23$, $p < 0.001$; reproductive traits: $r = 0.15$, $p < 0.05$; Mantel test) between populations of *C. defloratus.*

DISCUSSION

Quantitative genetic variation

The genetic variation of several quantitative traits within populations (evolvability) and of the principle component LEAFSIZE increased with centrality, but the genetic variation of most traits was not related to the range position, longitude or size of the populations. The increase in genetic variation of some traits with centrality is in line with the predictions of the ACM (Sagarin & Gaines 2002, Eckert et al. 2008), and with the increase in molecular genetic variation with centrality in *C. defloratus* (see Chapter 4). However, the genetic variation of none of the quantitative traits was significantly related to molecular genetic variation. This confirms the conclusions of the review by Reed & Frankham (2001) and the results of later studies (Steinger et al. 2002, Gravuer 2005, Navarro et al. 2005) that variation in molecular markers is a poor predictor of variation in quantitative traits. Many factors may contribute to a weak correlation between molecular and quantitative genetic variation, in particular the fact that molecular markers are assumed to be selectively neutral whereas genetic variation in quantitative traits will be influenced by natural selection (Reed & Frankham 2001).

Genetic theory predicts that genetic variation in both neutral molecular markers and quantitative traits should be reduced in small populations due to genetic drift (Eckert et al. 2008). However, while neutral marker diversity in *C. defloratus* significantly increased with population size (Chapter 4), genetic variation in none of the 18 studied quantitative traits or the four principle components was positively related to the size of the 32 study populations. While the negative effect of small population size on quantitative genetic variation is well supported by the results of experiments (Willi et al. 2006), most of the few field studies that have investigated the relationship between quantitative genetic variation and population size did not support the prediction of lower quantitative variation in small populations (Rice & Mack 1991, Widén & Andersson 1993, Waldmann & Andersson 1998, Meyer & Allen 1999, Podolsky 2001, Waldmann 2001, Gravuer et al. 2005). However, several of these studies had low power due to the small number of populations studied (Willi et al. 2006). In contrast, a study of 13 populations of the rare plant *Ranunculus reptans* found that genetic variation of quantitative traits was significantly reduced in small populations (Willi et al. 2007). Several factors may contribute to the mostly weak correlations found in field studies between census

size and quantitative genetic variation (Willi et al. 2006): Small populations may have declined only recently and differences in census size may thus only be weakly related to differences in effective population size, genetically impoverished populations may already have become extinct, selection may influence the patterns of quantitative genetic variation, and gene flow may increase variation in small populations. Of these factors, selection is the most likely to affect quantitative variation in *C. defloratus*, as there is a significant relationship between neutral marker diversity and census size (Chapter 4).

It has been suggested that the evolvability of life-history traits is typically higher than that of morphological traits (Hansen et al 2011). This was only partly supported by the results of our study. Among the genetically most variable traits were the fitness-related traits rosette number, leaf number and capitulum number, but also the purely morphological traits leaf incisiveness and leaf-capitulum distance.

Genetic differentiation in quantitative traits among populations

For all but one of the vegetative traits and for two principle components Q_{ST} was higher than Phi_{ST}, and for most of the traits the difference was significant, indicating that differentiating selection has contributed to the differentiation between populations. The pattern was less consistent for reproductive traits, but the Q_{ST} was higher than Phi_{ST} for four of the nine traits investigated, although significantly so only for one of them, due to large confidence intervals. Large confidence intervals have been found in many studies of quantitative genetic differentiation (e.g. Merilä & Crnokrak, 2001; O'Hara & Merilä, 2005; Martin et al., 2008).
Our finding of higher differentiation between populations in quantitative traits that may be under selection than in putatively neutral molecular markers is in line with the conclusions of recent reviews (Merilä & Crnokrak 2001, McKay and Latta 2002, Leinonen et al. 2008, Lamy et al 2012) that Q_{ST} is typically larger than Phi_{ST} respectively F_{ST} (McKay & Latta 2002, O'Hara & Merilä, 2005).

Further support for effects of differentiating selection on quantitative traits in *C. defloratus* is provided by the clinal variation of many trait means with the range position (centrality) or longitude east of populations (see Whitlock 2008). There was a very close correspondence between the Q_{ST} of a trait and clinal variation in trait means: traits which had a high Q_{ST} were also the ones that showed clinal variation. Both centrality and longitude are related to climate variables; the centrality gradient is a gradient of decreasing latitude and increasing altitude, but also of decreasing mean annual temperature ($r = -0.76$) and increasing annual precipitation

(r = 0.95), while with longitude east the difference between the mean temperature of the warmest and coldest month (r = 0.83) and that between the precipitation of the wettest and driest month increases (r = 0.56, all $p < 0.001$), indicating that longitude is related to the continentality of a site. The observed clines in trait means thus suggest that the differentiation between populations may be adaptive.

Overall, the quantitative genetic differentiation between populations of *C. defloratus* increased with their geographical distance, based both on vegetative and reproductive traits. However, the slopes of these relationships were much lower in peripheral than in central and intermediate populations, and as a consequence the mean differentiation between peripheral populations adjusted for geographic distance was also lower. This pattern is in sharp contrast to the pattern of genetic isolation by distance found for neutral markers. Marker based divergence between populations of *C. defloratus* increased much more strongly with distance for peripheral than for central and intermediate populations (Chapter 4).

The marker based pattern is in agreement with the predictions of the ACM that stronger isolation and lower population size will result in stronger differentiation between peripheral populations through genetic drift. However, the contrasting pattern found for quantitative genetic differentiation indicates that habitat conditions and thus selective forces are more similar for the peripheral than for the other populations.

Most previous studies did not find a relationship between quantitative genetic differentiation and geographic distance between plant populations (Petit et al. 2001, Steinger et al. 2002, Jolivet and Bernasconi 2007, Gravuer et al 2005, Korbo et al. 2012). However, due to the small number of populations studied, the power of the studies by Petit et al. (2001) and Jolivet & Bernasconi (2007) was low. Two studies, on *Nassella pulchra* (Knapp & Rice 1998) and *Primula sieboldii* (Yoshioka et al. 2007), found a significant increase of quantitative differentiation with geographical distance. In both studies, as in our study, the maximum distance between populations was large, which should increase the likelihood of strong environmental differences and thus different selection regimes between populations.

In *C. defloratus*, the quantitative genetic differentiation between populations based on vegetative traits was related to the molecular genetic differentiation, but not that based on reproductive traits. Significant positive correlations between the two types of genetic differentiation have been interpreted as an indication that drift has contributed to population divergence (Willi et al. 2007). However, if both types of variation increase with geographical

distance, such a correlation could also arise because of increasing divergence of selective forces with distance.

The results of previous studies of the relationship between quantitative and molecular genetic differentiation have not been consistent. Of the studies that compared more than six populations, those on *Picea abies* (Lagercrantz & Ryman 1990), *Senecio vulgaris* (Steinger et al. 2002) and *Primula sieboldii* (Yoshioka et al. 2007) found a correlation between quantitative and molecular genetic distance, while in *Liatris scariosa* there was a correlation for some quantitative traits, but not for others (Gravuer et al. 2005). In contrast, the quantitative and molecular genetic differentiation between populations was not related in *Nassella pulchra* (Knapp & Rice 1998). Our results indicate that conflicting results may result from the choice of traits studied.

CONCLUSIONS

The ACM predicts that because of stronger genetic drift, populations at the range margin are genetically less diverse and more differentiated than those at the range centre (Sagarin & Gaines 2002, Eckert et al. 2008). While the variation in molecular markers for *C. defloratus* was in line with the predictions of the ACM, genetic variation in most quantitative traits was not lower in peripheral than in central populations, and genetic differentiation between populations in these traits was lower. The contrasting patterns found for the variation in quantitative traits in *C. defloratus* are likely due to selection, as several lines of evidence indicated that populations of *C. defloratus* show adaptive differentiation in quantitative traits. Genetic differentiation between populations in many quantitative traits (Q_{ST}) was higher than that in molecular markers (Phi_{ST}), and trait means differed among populations and were related to environmental gradients. The weak correlations we found between the variation in quantitative traits and marker diversity in populations confirm the conclusion that the variation in molecular markers is a poor predictor of genetic variability of genetic variation in quantitative traits that may be important for the adaptation of species to changing conditions (see Frankham et al. 2001).

Outside of its distribution centre in the Alps, *C. defloratus* is a rare species that is not yet considered to be threatened, but included in the advance warning list of the states of Hesse (Buttler et al. 1997) and Baden-Württemberg (Breunig & Demuth 1999). The conservation value of peripheral populations is a matter of debate (Lesica & Allendorf 1992, 1995, Van Rossum et al. 2003). Our results indicate that peripheral populations, even if their genetic

variability in neutral markers is reduced, do not necessarily suffer from genetic erosion in traits under selection that are important for the evolvability of a species, and that the evolvability of small populations may also be high. The clines in trait means along the centrality gradient indicate that peripheral populations of *C. defloratus* are adapted to the conditions at the range margin. As the populations at the northern range margin grow at the lowest altitude and represent the receding edge in the face of climate change (Hampe & Petit 2005), they are likely to contain alleles that may become important for the adaptation of the species to a warmer climate elsewhere. However, as the populations outside of the distribution centre are isolated from each other and dispersal is poor, it is unlikely that important alleles will spread.

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SUMMARY

The aim of this thesis was to test several of the predictions of the abundant centre model (ACM) by comparing central and peripheral populations of the model species *Carduus defloratus* along a gradient from the centre towards the periphery of the distribution of the species. The ACM predicts that because of increasingly unfavorable and stressful conditions populations become less frequent, smaller, and less dense towards the range edges. Further predictions for peripheral populations derived from the model include lower reproduction of organisms, higher temporal variability of demographic transitions and of population growth rates, higher genetic differentiation among populations and lower within-population genetic diversity.

Most of these predictions of the ACM were supported in *C. defloratus*. The size of populations, their density and reproduction, but also the proportion of seeds damaged by insects decreased from the distribution centre of the species towards the periphery. The number of flowering plants in a population influenced all components of reproduction. Plants in large populations initiated more seeds, aborted less seeds, and produced more and larger seeds per plant. This indicates pollen limitation and increased inbreeding in small, peripheral populations. The strongly reduced reproduction in combination with the lack of suitable, open rocky habitats and poor dispersal of the seeds limits the abundance of *C. defloratus* towards its northern range limit.

Demographic studies in 14 populations of *C. defloratus* along the central-peripheral gradient did not indicate significant differences between peripheral and central populations in the asymptotic growth rate (λ) of the populations, of the temporal variability in λ, or in the extinction risk of populations of a certain size. However, the variability of several demographic transitions like seedling survival and stasis of vegetative plants decreased toward the periphery, but changes in these transitions compensated each other. These results are in contrast to the hypothesis of increasing demographic variability towards the periphery of the distribution of a species and an increased extinction risk of peripheral populations. Both population types differed significantly in particular demographic transitions, the contribution of particular transitions to λ, their stage structure, and the life span of plants. The fact that demographic features of *C. defloratus* showed clinal variation related to gradients in centrality and thus climate, suggest that it might be possible to predict general demographic features for individual populations based on their environment.

An analysis of the genetic variability and genetic structure of 78 populations of *C. defloratus* based on AFLPs indicated that genetic variability within populations decreased towards the

periphery whereas the genetic differentiation between populations increased. A strong increase of genetic differentiation between pairs of populations with their geographic distance (isolation by distance) indicated gene flow between neighbouring populations. The pattern found is likely to have been formed during the last glaciation, because the populations of *C. defloratus* outside of the Alps are very isolated and gene flow between them has been very unlikely for a long time. This result together with the small effect of current population size on genetic variability indicates that the population genetic structure of the long-lived species is mainly influenced by historical processes. Like the genetic diversity of molecular genetic markers, that of several quantitative traits decreased from the distribution centre towards the range margin. However, in contrast to the molecular genetic differentiation, the differentiation in quantitative traits did not increase towards the periphery. Quantitative and molecular genetic diversity were not correlated significantly and correlations between quantitative and molecular genetic differentiation were either weak or not significant.

The quantitative genetic differentiation of several traits (Q_{ST}) between 32 populations of *C. defloraut*s was stronger than the differentiation between molecular markers (Phi_{ST}) and some traits showed clinal variation with regard to environmental gradients, indicating that divergent selection acts on quantitative traits. The clinal variation in quantitative traits indicates that the observed differences are adaptive. The northern peripheral populations are likely to contain alleles that may become important for the adaptation of the species to a warmer climate. The northern range limit of *C. defloratus* rather results from lack of suitable open, rocky habitats than from poor adaptation to climatic conditions in the north.

Altogether, the combination of different studies on a single model species and the results of this thesis contribute to a better understanding of the role of current and historic, and of demographic and genetic processes for the differentiation between central and peripheral populations. Moreover, these studies contribute to the discussion about the conservation value of small, peripheral populations in a time of climatic change.

ZUSAMMENFASSUNG

Das Ziel dieser Dissertation war es, die Vorhersagen des "Abundant Center Models" (ACM) durch einen Vergleich von Populationen von *Carduus defloratus* entlang eines Gradienten vom Zentrum zur Peripherie des Verbreitungsgebietes zu testen. Das ACM sagt voraus, dass aufgrund von zunehmend ungünstigen Bedingungen zum Rand des Verbreitungsgebietes hin Populationen kleiner und weniger dicht werden. Weitere vom ACM abgeleitete Vorhersagen sind, dass periphere Populationen eine geringere Reproduktion der Individuen aufweisen, und dass die zeitliche Variabilität der demographischen Übergänge sowie der Wachstumsraten der Populationen größer ist. Aufgrund der geringeren Größe der Populationen und ihrer stärkeren Isolation voneinander, sollte außerdem die genetische Variabilität in Populationen zur Peripherie hin abnehmen und ihre Differenzierung zunehmen.

Die meisten dieser Vorhersagen des ACM trafen auf *C. defloratus* zu. Die Größe der Populationen, die Dichte der Pflanzen und ihre Reproduktion, aber auch der Anteil durch Insekten geschädigter Samen nahm vom Zentrum zur Peripherie des Verbreitungsgebietes ab. Die Anzahl Pflanzen in einer Population beeinflusste alle Komponenten der Reproduktion der Pflanzen. Pflanzen in großen Populationen setzten mehr Samen an, abortierten weniger Samen und produzierten mehr und größere Samen. Dies deutet auf eine verringerte Bestäubung und auf stärkere Inzucht in den kleineren peripheren Populationen hin. Die stark reduzierte Reproduktion, in Kombination mit dem Mangel an geeigneten, offenen Felshabitaten und der geringen Ausbreitungsfähigkeit der Samen, begrenzt offenbar die Abundanz der Art zur nördlichen Verbreitungsgrenze hin.

Demographische Studien in 14 Populationen von *C. defloratus* entlang des Zentralitätsgradienten zeigten keine signifikanten Unterschiede zwischen peripheren und zentralen Populationen in der asymptotischen Wachstumsrate (λ) der Populationen, der zeitlichen Variabilität in λ, oder des Aussterberisikos von Populationen einer bestimmten Größe. Allerdings nahm die Variabilität einiger demographischer Übergänge, wie die des Überlebens der Keimlinge und der Stasis vegetativer Pflanzen, zur Peripherie hin ab. Diese Ergebnisse widersprechen der Hypothese, dass die Variabilität der Demographie zur Peripherie der Verbreitung einer Art zunimmt und periphere Populationen deshalb stärker gefährdet sind. Die beiden Typen von Populationen unterschieden sich in den einzelnen demographischen Übergängen, deren zeitlicher Variabilität, den Beiträgen der einzelnen Übergänge zu λ, in ihrer Stadienstruktur und in der Lebensdauer der Pflanzen. Allerdings kompensierten sich Unterschiede in verschiedenen Übergängen stark, so dass die Variabilität in λ nicht unterschiedlich war. Das Ergebnis, dass die demographischen Eigenschaften von *C. defloratus* in Beziehung zur Zentralität einer Population und damit zu klimatischen

Gradienten stehen, gibt Anlass zu der Hoffnung, dass es möglich sein könnte, demographische Eigenschaften von einzelnen Populationen einer Art aufgrund der Umweltbedingungen vorherzusagen.

Eine Analyse der genetischen Struktur und Variabilität von 78 Populationen von *C. defloratus* mit AFLP-Markern zeigte, dass die genetische Variabilität innerhalb der Populationen zur Peripherie hin abnahm, während die Differenzierung zwischen Populationen zunahm. Die genetische Differenzierung zwischen Populationen nahm mit ihrer Entfernung voneinander stark zu, was auf Genfluss in der Vergangenheit (möglicherweise während der letzten Eiszeit) hindeutet, da die Populationen von *C. defloratus* außerhalb der Alpen seit langem isoliert sind und aktueller Genfluss sehr unwahrscheinlich ist. Zusammen mit dem geringen Effekt der aktuellen Größe der Populationen auf die genetische Variabilität zeigt dies, dass die genetische Struktur der Populationen der langlebigen Art *C. defloratus* vor allem durch historische Prozesse bedingt ist. Wie die genetische Variabilität molekularer Marker, nahm auch diejenige einiger weniger quantitativer Merkmale vom Zentrum zur Peripherie hin ab. Die quantitativ-genetische Differenzierung zwischen Populationen nahm im Gegensatz zu derjenigen molekularer Marker nicht zur Peripherie hin zu. Quantitativ- und molekular-genetische Variabilität waren nicht signifikant korreliert und nur die genetische Differenzierung quantitativer vegetativer Merkmale war mit jener der molekularer Merkmale korreliert.

Die genetische Differenzierung in verschiedenen quantitativen Merkmalen (Q_{ST}) zwischen 32 Populationen von *C. defloratus* war deutlich stärker als die zwischen molekularen Markern (Phi_{ST}) und verschiedene Merkmale zeigten klinale Variation in Beziehung zu Umweltgradienten. Die weist auf den Einfluss divergenter Selektion und auf lokale Adaptation hin. Die nördlichen peripheren Populationen sind daher besonders wertvoll für die Anpassung der Art an ein wärmeres Klima. Der nördliche Verbreitungsrand von *C. defloratus* lässt sich eher durch einen Mangel an offenen, felsigen Habitaten als durch eine zu geringe Anpassungsfähigkeit an die klimatischen Bedingungen im Norden erklären.

Insgesamt tragen die verschiedenen Studien an einer Modellart zu einem besseren Verständnis des Einflusses aktueller und historischer, sowie demographischer und genetischer Prozesse auf die Unterschiede zwischen zentralen und peripheren Populationen bei. Darüber hinaus sind die Studien ein Beitrag zur Diskussion über den Wert kleiner, peripherer Populationen für den Naturschutz in einer Zeit des Klimawandels.

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Education and professional experience

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01/2012 – 06/2012	Phd-Grant of the Marburg Research Academy (MARA)
06/ 2006 – 12/ 2008	Collaboration on the DFG proposal "The ecology and genetics of central and peripheral populations of *C. defloratus*" with Prof. Dr. Diethart Matthies. Sampling leaves and seeds of *C. defloratus*, preparation and execution of common garden experiments and managing crossing experiments of *C. defloratus* with Jeremias Becker (diploma student). Development of AFLP primer combinations for *C. defloratus*, *Thapinantus oleifolius* and three *Acacia* species.
08/2005 – 12/2008	Research assistant in the Department of Ecology (Laboratory of Prof. Dr. Diethart Matthies) at the Faculty of Biology, Philipps-Universität Marburg, Germany, research project: Genetic diversity, clonal diversity, and fine-scale spatial genetic structure of the rare and endangered *Gentianopsis ciliata* in Germany and Luxemburg: Search for populations of *G. ciliata* in Central Europe, test of allozyme reactions in *G. ciliata,* establishing the method of amplified fragment length polymorphism (AFLP) in the Department of Ecology, development of AFLP primer combinations for *G. ciliata* and analysis.
10/1999 – 04/ 2005	Studies in biology at the Philipps-Universität Marburg (Germany). Main subjects: general ecology and animal ecology, conservation biology, and geography. Diploma thesis at the Faculty of General Ecology and Animal Ecology (Laboratory of Prof. Dr. Roland Brandl), Philipps-Universität Marburg, Germany: "Population genetic analysis of the rose hip fly *Rhagoletis alternata* (Diptera: Tephritidae)".
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PUBLICATIONS

Published papers and book chapters

(1) Vaupel A, Matthies D (2012) Abundance, reproduction, and seed predation of an alpine plant decrease from the centre towards the range limit. *Ecology*, 93, 2253-2262.

(2) Kohnen A, Brandl R, Fricke R, Gallenmüller F, Klinge K, Köhnen I, Maier, Oberwinkler F, Ritz C, Speck T, Theissen G, Tscharntke T, Vaupel A, Wissemann V (2010) Radiation, biological diversity and host-parasite interactions in wildroses, rust fungi and insects. Pages 215-238 *in* M Glaubrecht, H Schneider, editors. *Evolution in Action – Adaptive Radiations and the Origin of Biodiversity*. Springer Verlag. Berlin, DE.

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Talks and poster presentations

(1) Vaupel A (2011) Geographical variation in population biology and genetic structure of the alpine thistle, *Carduus defloratus* (Seminar in plant ecology, Botanical Institute, University of Basel, **(Invited talk**).

(2) Vaupel A and Matthies D (2011) Genetic structure of peripheral and central populations of *Carduus defloratus,* 41th Annual Meeting of the Ecological Society of Germany, Austria and Switzerland (GFOE), Oldenburg (Talk).

(3) Vaupel A and Matthies D (2011) Population structure and genetic diversity of central and peripheral populations of *Carduus defloratus*, 96th Annual Meeting of the Ecological Society of America (ESA), Austin, Texas (Talk)

(4) Vaupel A and Matthies D (2011) Population structure and genetic diversity of *Carduus defloratus* from the centre to the periphery of its distribution, 23th Annual Plant Population Biology Conference, Oxford (Talk).

(5) Vaupel A and Matthies D (2010) A comparison of central and peripheral populations of *Carduus defloratus*, 40th Annual Meeting of the Ecological Society of Germany, Austria and Switzerland (GFOE), Gießen (Talk).

(6) Vaupel A (2005) Population genetic analysis of the rose hip fly *Rhagoletis alternata* (Diptera: Tephritidae), Colloquium at the Department of Agroecology, Georg-August-Universität Göttingen **(Invited talk).**

(7) Vaupel A and Matthies D (2009) Adaptive population differentiation between peripheral and central populations of *Carduus defloratus*, 22th Annual Plant Population Biology Conference, Bern (Poster).

(8) Vaupel A, Becker J, Matthies D (2008). Differences between peripheral and central populations of the disjunctly distributed relict species *Carduus defloratus*, 21th Annual Plant Population Biology Conference, Luxemburg **(Poster award).**

(9) Vaupel A und Matthies D (2007) The genetic structure of populations of the threatened calcareous grassland species *Gentianella ciliata*. 37th Annual Meeting of the Ecological Society of Germany, Austria and Switzerland (GFOE), Marburg (Poster)

(10) Vaupel A, Becker J, Matthies D (2007) Differences between central and marginal populations of the glacial relict species *Carduus defloratus*, Symposium Phylogeography and Conservation of Postglacial Relicts, Luxemburg (Poster).

(11) Vaupel A, Kohnen A, Brandl R (2007) Low genetic differentiation between populations of the European rose hip fly *Rhagoletis alternata*. 37th Annual Meeting of the Ecological Society of Germany, Austria and Switzerland (GFOE), Marburg (Poster)

(12) Vaupel A, Lehmann K, Wissemann V, Brändle M, Tscharntke T, Brandl R (2005) Genetic differentiation between populations of the European rose hip fly *Rhagoletis alternata* (Diptera: Tephritidae). 98th Annual Conference of the German Zoological Society (DZG), Bayreuth (Poster).

(13) Vaupel A, Klose M, Brändle M, Brandl R (2004) Morphological and genetic patterns of Central European spring snail species *Bythinella* (Gastropoda: Prosobranchia). 97th Annual Conference of the German Zoological Society (DZG), Rostock (Poster).

Erklärung

ich versichere, dass ich meine Dissertation

"**The ecology and genetics of central and peripheral populations of *Carduus defloratus***"

selbständig, ohne unerlaubte Hilfe angefertigt und mich dabei keiner anderen als der von mir ausdrücklich bezeichneten Quellen und Hilfen bedient habe.

Die Dissertation wurde in der jetzigen oder einer ähnlichen Form noch bei keiner anderen Hochschule eingereicht und hat noch keinen sonstigen Prüfungszwecken gedient.

Marburg (Lahn), den 04.01.2013 _____

Andrea Vaupel